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PUSA







# ANNALS OF BOTANY

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AND OTHER BOTANISTS

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# The Meiotic Divisions in the Pollen Mother-cells of *Malva sylvestris*.

BY

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With Plates I and II.

THIS paper is the result of an investigation of meiosis in the pollen mother-cells of *Malva sylvestris*. As with other plants of the Malvaceae, perfect fixation was difficult to obtain and the material consequently did not prove suitable for a detailed study of all the meiotic phases. Division stages, however, showed good fixation, and particular attention was therefore given to obtaining chromosome counts and establishing the haploid number, which had not previously been determined for this species.

## MATERIAL AND METHODS.

Flower buds of *M. sylvestris* were collected from plants growing in Chelsea Physic Gardens in June 1929. The fixing fluids employed were Carnoy, weak chrome acetic, Bouin, and Allen's modification of Bouin. The sepals were removed from the buds to facilitate penetration of the fixative, and at each collection an exhaust pump was used. All the material was treated with the paraffin method, sectioned at thicknesses from 10–16  $\mu$ , and stained with Heidenhain's iron alum haematoxylin.

As has been observed by previous authors investigating plants of the Malvaceae—Denham (6), Beal (3)—the material proved capricious as regards fixation, the quality of which showed considerable variation with any one of the fluids used.

## OBSERVATIONS.

The young anthers of *M. sylvestris* are packed closely together with no apparent orientation. Cross sections or longitudinal sections of the buds therefore show loculi cut at many different angles. The pollen mother-cells are extremely large, and in the cross-section of a loculus there is frequently only one mother-cell to be seen with the tapetal cells closely

surrounding it. The nucleus and nucleoli of the resting mother-cells are also very large. Aggregations of dense-staining granules are sometimes present in the cytoplasm (Pl. I, Fig. 1). Similar aggregations of granules have occasionally been observed round the interkinetic nuclei and during homotypic metaphase. Their significance is not understood, and they are by no means a constant cytoplasmic feature.

In the resting nucleus and very early stages of meiosis all the stainable material is located in the nucleolus. The nuclear cavity contains very delicate indistinct strands of material of granular appearance. At the onset of prophase this lightly staining material aggregates at one side of the nucleus whilst remaining in contact with the nucleolus (Pl. I, Fig. 2). This stage is obviously that of synizesis but it is impossible to distinguish any threadlike portions until loosening of the synizetic knot occurs. The nucleolus is frequently papillate and shows the extrusion of buds into the granular mass lying beside it. As the synizetic knot begins to loosen definite portions of thread can be seen at its periphery. At this stage the thread could not be followed with accuracy for any considerable length, nor was it possible to determine whether it was a single or a double structure. Very definite connexions exist between the spireme and the nucleolus. One or more dark-staining nucleolar bodies are present, either situated at the periphery of the large vacuolate nucleolus (Pl. I, Fig. 3) or, occasionally, attached to an extruded bud of nucleolar material (Pl. I, Fig. 4). The spireme is clearly connected to these dark nucleolar bodies. That portion of it nearest the body may be thickened, indicating a possible outward flow of material at this time of spireme formation.

As loosening of the synizetic knot proceeds, the threadwork becomes distributed throughout the nuclear area. None of the preparations examined showed the presence of a continuous spireme, and in all the stages leading to diakinesis the portions of thread have a decidedly granular appearance. This is due to the distribution of dark globules of substance on a more delicate and fainter staining thread. Then delicate 'beaded' portions of thread soon show side-by-side association over the entire nuclear area. It was impossible to discern such parallel pairing of all the mass of fine threads. Their 'beaded' appearance and the slight staining capacity of the connexions between the granules made them difficult to trace, and rendered distinction of individual strands impossible in a tangled mass. There were, however, sufficient examples of parallel association recognizable in all the well-fixed nuclei examined at this stage to permit of the conclusion that the mode of chromosome pairing is parasynaptic. Pl. I, Fig. 5, was drawn from a typical nucleus at this stage and shows only those portions of threads where the side-by-side arrangement could be seen. The granular threads which could not be followed, and which often appeared to be a somewhat reticulate mass, are not depicted.

These parallel strands twist round one another and form the very much thickened bivalent threads. Pl. I, Fig. 6 shows a tangential view of a nucleus where the formation of some of the bivalents is clearly seen. These thickened threads also appear beaded, as would be expected from the twisting together of their irregular components. Two univalents not yet closely associated are seen on the right in this nucleus, while separation of the univalent portions is seen at one or more points in each of the formed bivalents. The difference in thickness of univalent and bivalent strands is quite marked. One bivalent shown in this figure is still in connexion with a nucleolar bud. The nuclear membrane is very delicate, and often scarcely discernible during these stages. It is, however, a known fact that the definition of this membrane depends greatly on the fixing fluid employed, and possibly its appearance in this preparation is due to technique.

### *Diakinesis.*

Comparatively few stages of diakinesis were found in the material examined. This phase must be passed over very rapidly, as, apparently, are all the subsequent meiotic stages in *M. sylvestris*. Out of nearly a hundred buds sectioned, only four showed any considerable number of division figures. The collections of material were made on bright sunny mornings under conditions normally suitable for active cell-division.

Extreme contraction of the bivalent threads occurs immediately prior to diakinesis, and the appearance of the nuclei in this stage suggests that a portion of the 'thread mass' is not included in the chromosomes. This remaining portion probably corresponds to the linin threads described in *Lavatera* by Byxbee (4), which, according to her account, contribute to spindle formation. In *M. sylvestris* these remaining threads form a faintly staining mass of somewhat reticulate structure around the nucleolus, while the contracted bivalents take up a peripheral position in the nucleus (Pl. I, Fig. 7).

At this time a marked change occurs in the appearance of the cytoplasm by the formation of the 'perinuclear zone'—a dense granular layer immediately surrounding the nucleus. This zone was described in detail by Byxbee in *Lavatera* and has been noted by all investigators of meiosis in Malvaceous plants. When it first appears this zone is not clearly delimited at its outer edge from the more reticulate peripheral cytoplasm, but seems to merge gradually into the latter (Pl. I, Fig. 8). Later on it becomes a very definite deep-staining zone of considerable width. The perinuclear zones are characteristic of the heterotypic and homotypic divisions and facilitate the detection of division stages under a low magnification.

The nucleolus disappears during the later stages of diakinesis. Its

disappearance must occur very rapidly, as uncut nuclei in which the form and distribution of the chromosomes is similar may contain a large nucleolus of considerable staining capacity, or show no sign at all of nucleolar material. The nuclear membrane also breaks down at this time. Pl. II, Fig. 9, shows a nucleus in late diakinesis. There is no nucleolar material, and only portions of membrane can be seen. Seventeen bivalent chromosomes are in the nuclear area, two shown displaced in the perinuclear zone, while two more were further displaced in the cytoplasm but are not figured. A number of faintly-stained indistinct strands of substance are present in the nucleus in addition to the bivalent chromosomes. These are the remains of the 'thread mass' which were not utilized at chromosome formation—i.e. the linin threads of Byxbee. In all diakinetic nuclei a quantity of this faintly-stained substance is present as well as the bivalent chromosomes. At diakinesis the nuclei attain their maximum size.

#### *Spindle formation.*

In the present investigation no attempt was made at a detailed study of the mode of spindle formation. The amount of material available showing diakinesis was insufficient for an accurate study of this phenomenon.

Byxbee (4) describes the cytoplasm of *Lavatera* as composed of two constituents, a fibrous network and a granular substance. The accumulation of the granular substance about the nuclear wall leads to the formation of the characteristic 'perinuclear zone' which entirely obscures the reticulum in that part of the cytoplasm. A little later the fibres of the reticulum immediately outside the nuclear membrane come into clearer view, as though the granular matter had been used up in that region. There is then a radially arranged felt of cytoplasmic fibres about the nucleus. As the nuclear membrane disappears, these cytoplasmic fibres grow into the nuclear cavity and mingle in an interwoven mass with the 'linin threads' of the nucleus. This mass of fibres, of both cytoplasmic and nuclear origin, gives rise to the multipolar spindle. The continued straightening-out and converging of the fibres brings the chromosomes on to the equatorial plate of a bipolar spindle.

Beal (3) states that the perinuclear zone performs an important function in spindle formation in the heterotypic division in *Gossypium*. There is no radially arranged felt of fibres about the nucleus as observed by Byxbee in *Lavatera*, but, as the nuclear membrane disappears, fibres seem to grow into the nuclear cavity from the inner portion of the perinuclear zone. These become attached to the chromosomes. Other fibres can be seen connecting the chromosomes, but their origin is uncertain.

My observations on *M. sylvestris* indicate that spindle formation is as described by Beal for *Gossypium*. There is no evidence of a felt of cyto-

plasmic fibres between the nuclear cavity and the perinuclear zone, and the poles of the early multi- or tripolar spindles are in all cases seen in direct contact with the perinuclear zone, suggesting their formation from its inner edge. The faintly staining strands of material present in nuclei at late diakinesis may be taken to correspond to the linin threads of Byxbee and possibly contribute to spindle formation. Possibly some such material is also present in *Gossypium* and forms the fibres whose origin Beal was not able to ascertain.

#### *Heterotypic division.*

When the bivalent chromosomes become grouped on the equatorial plate of the bipolar spindle they are extremely contracted and their bivalent nature usually unrecognizable. Counts were made from polar views of the equatorial plate on which the small chromosomes usually lie free from one another in one plane, or in slightly different planes but readily distinguishable on careful focusing.

There are constantly twenty-one chromosomes present at heterotypic metaphase (Pl. II, Fig. 10). These consist of nineteen bivalents and two unpaired univalents. They are nearly all of similar ovoid shape, but show considerable variation in size. The splitting of the bivalents into their component members at anaphase does not take place simultaneously in any one chromosome group. This fact caused great variation in the chromosome counts made on the equatorial plate as there is no very rapid separation of univalents after the anaphasic split has occurred. Pl. II, Fig. 11, shows a group of seventeen compact bivalent chromosomes, four univalents, and two bivalents in which the beginning of the anaphasic split is suggested. A polar view of early anaphase (Pl. II, Fig. 12) shows nineteen univalents still closely associated in pairs, but in two distinct planes, and two univalents of larger size. The total chromosome number is thus forty. The fact that these two larger chromosomes are univalents and not late-splitting bivalents is proved by the occurrence of twenty chromosomes in each daughter nucleus at homotypic division. The phenomenon of exclusion of chromosomes due to 'lagging' or any other cause was never observed at either first or second division. The univalent chromosomes presumably pass undivided one to each pole.

The perinuclear zone completely surrounds the spindle area during heterotypic division (Pl. II, Fig. 13), but towards telophase it loses its staining capacity to a large degree. It then encroaches upon the spindle area and forms a zone around each daughter nucleus.

#### *Interkinesis.*

Nuclear membranes are formed round the daughter nuclei at interkinesis, and one to four spherical nucleoli of varying sizes are usually

present. The individual chromosomes are quite unrecognizable (Pl. II, Fig. 14). Several preparations showing interkinetic nuclei revealed no sign of a perinuclear zone, but if the section were more deeply stained this was quite clearly seen. It would appear that the staining capacity of this granular zone is at its lowest during interkinesis, and is most pronounced during metaphase.

#### *Homotypic division.*

The homotypic chromosomes are characterized by their extremely small size. The formation of the homotypic spindles and the arrangement of the chromosomes upon the equatorial plate apparently takes place very rapidly. The spindles may lie at almost any angle to one another and the nuclear area of each homotypic group is surrounded by a well-marked perinuclear zone (Pl. II, Fig. 15). No irregularities in chromosome behaviour were noted, and in homotypic anaphase twenty chromosomes pass regularly to each pole. On account of their small size the chromosomes usually lie quite free from one another and many counts were obtained from polar views of groups at this stage (Pl. II, Fig. 16). Occasionally the full number of chromosomes could be clearly distinguished in a side view of the homotypic spindle (Pl. II, Fig. 17). The chromosomes soon lose their identity on reaching the spindle poles, and normal daughter nuclei with membranes and one or more nucleoli are soon established.

As in the heterotypic division, the perinuclear zones lose their staining capacity at the approach of telophase, but at the reconstitution of the daughter nuclei the granular matter again becomes conspicuous and covers most of the cytoplasmic area, leaving only a small peripheral zone unoccupied (Pl. II, Fig. 18). The granular substance then collects about the four nuclei as shown in Pl. II, Fig. 19. Striations are seen in the pale-staining cytoplasm outside the perinuclear zones. These may be delicate spindle fibres which are often formed uniting tetrad nuclei prior to quadripartition of pollen mother-cells or may possibly represent the lines of flow along which the granular substance of the cytoplasm has travelled while forming the perinuclear zones. Their delicate appearance would indicate that the latter explanation is the more probable.

#### *Cell-wall formation.*

It was noted by Beal in *Gossypium* that at the time of the loosening of the synizetic knot the loculi of the anthers increase in size. The walls of the pollen mother-cells extend and remain attached to the tapetal cells, but the protoplasts do not increase in size proportionately. This results in the separation of the protoplast from the mother-cell walls. After this separation the protoplast forms a new independent wall about itself. These

new walls show great variation in thickness around different mother-cells at similar stages of development.

Similar events have been noted in *M. sylvestris* though the preparations were not specially stained for the elucidation of the mode of cell-wall formation. Diakinesis is the earliest nuclear stage at which an independent homogeneous wall is observed around the protoplast while the pollen mother-cell wall lies between it and the tapetum. This wall does not increase very much in thickness during heterotypic and homotypic divisions, and shows great variation in width at the time of tetrad formation. Partition of the mother-cell protoplast appears to take place by furrowing: in no case was any attempt at cell-plate formation observed.

#### DISCUSSION.

On account of their economic importance, species of *Gossypium* have received more cytological study than other Malvaceous plants, though the work on this genus is not extensive. The more important papers of Cannon (5), Balls (1), and Denham (6 and 7) are briefly reviewed by Beal (3) and will not, therefore, be discussed separately here.

The heterotypic prophase stages in *M. sylvestris* show marked resemblance to those observed by Beal in strains of *Gossypium barbadense* in which the method of chromosome pairing is shown to be parasynaptic. These results contrast, with those of Denham, who reported telosynaptic association in *G. barbadense* var. *maritima*. Despite the similar method of chromosome pairing in *M. sylvestris* and some strains of *G. barbadense* certain differences are apparent in the prophase stages. Beal reports that the parallelism of threads is obvious before the strands are pulled into the synizetic knot and that, as the knot loosens, loops of double threads are formed which disclose no free ends. This association of threads prior to synizesis is much earlier than is apparent in *M. sylvestris*, in which genus there is no evidence of continuity of the spireme. There is also definite connexion of the spireme with the nucleolus in *M. sylvestris*, while in *Gossypium* it is stated that the nucleolus lies free.

It is chiefly in the formation of bivalent threads by twisting of the strands about one another that *Gossypium* and *Malva* show resemblance. Many of the figures given by Beal illustrate exactly the same condition as shown in *Malva* (Pl. I, Fig. 6). He states that 'the strands composing the threads may be rather widely separated in places: in other places loosely twisted: in still others, so closely twisted and united as to give the appearance of a single, though thicker thread.' The bivalent chromosomes are formed by massing of the chromatic material in definite parts of the spireme. This results in lumped heavy stained masses with alternating thinner and more lightly stained regions in each strand. The strands segment at the thinner parts, i.e. the bivalents are formed by the direct

transverse segmentation of a heterogeneous bivalent spireme and not as a result of looping and folding.

The formation of bivalents could not be followed in such detail in the material of *Malva* owing to the rapidity with which the stages are accomplished, but the parallel association of separate strands (Pl. I, Fig. 5), their twisting about one another to form double threads (Pl. I, Fig. 6), and the complete absence of any looping stage seem sufficient to warrant the inclusion of *M. sylvestris* among those plants showing parasynaptic chromosome pairing.

#### *Chromosome number.*

Twenty is recorded as the haploid chromosome number for *M. moschata*, approximately 20 for *M. palmata*, and from 20 to 30 for *M. purilla* (Tischler, 9). Twenty is also the reduced number for *Lavatera thuringiaca* (Tischler, 9). Other Malvaceous plants investigated show considerable divergence from this number, having 13, 21, 26, 40, 48, and 72 as haploid numbers (Tischler, 9; Gaiser, 8). The last three numbers given are for species of *Hibiscus* determined by Youngman (10), who states that these figures indicate 'that in the genus *Hibiscus* the chromosomes are a simple multiplication of four as a factor, or more probably still, in the light of information gathered from the study of *Thespesia*, eight'. Youngman gives the numbers 8, 10, and 13 as haploid in *Thespesia populnea*, these various numbers being seen as the reduced number of chromosomes at different stages in the meiotic division. On the heterotypic equatorial plates thirteen bodies mass together at the centre as eight. On the homotypic equatorial plates ten and thirteen chromosome bodies appear respectively in the sister cells in the pollen tetrad, three nuclei containing ten chromosomes and one nucleus thirteen. In a footnote, Youngman reports that subsequent observations reveal the probability of the factorial number in *Thespesia* being six or seven rather than eight. This perhaps indicates errors in some of the previously determined counts.<sup>1</sup> His suggestion of eight as the fundamental number is, however, supported by comparison with numbers given for other plants of the Malvales, the families of which are closely allied taxonomically. Three plants of Tiliaceae are recorded by Tischler with the haploid numbers 8, 30–33, and about 80, and 8 and 16 are respectively the haploid and diploid numbers in *Theobroma cacao* of the Sterculiaceae. In a recent publication, Youngman (11) gives the numbers of 'chromosome bodies' determined for certain Hibisceae and allied

<sup>1</sup> Since this paper was prepared for publication, a further study by Youngman has appeared. (Ann. Bot. xlv., 211–227, 1931). His observations on the prophase of the nucleus of the pollen mother-cell of *Thespesia populnea* explain phenomena observed in the meiotic division. Both thirteen and eight chromosome bodies have been seen upon the equatorial plate at different times in the same cell.



plants in the somatic divisions of their root tips. For the members of the Hibisceae investigated the number is 26, and for *Guazuma tomentosa*, a plant with near affinities to *Theobroma*, 16.

The only haploid count of twenty-one is given for *Malvastrum capense* (Tischler, 9). Denham's investigations (7) on chromosome numbers in American, Egyptian, and Indian cottons show that the American and Egyptian varieties have twenty-six and the Indian varieties thirteen haploid chromosomes. Likewise, Banerji (2) observed thirteen haploid chromosomes in twenty-eight strains of Indian cottons differing considerably in morphological and other characters, and twenty-six chromosomes in four American cottons. Youngman's (11) counts for *Gossypium* species are in accordance with these results of Denham and Banerji.

From these determinations it appears that 8 (?), 10, and 13 may all represent fundamental chromosome numbers in the Malvaceous plants so far investigated.

#### SUMMARY.

1. The meiotic divisions in the pollen mother-cells are described for *M. sylvestris*.
2. One or more connexions exist between the nucleolus and spireme threads.
3. There is strong evidence favouring a parasynaptic interpretation of the mode of chromosome pairing.
4. A perinuclear zone, characteristic of Malvaceous plants, forms about the diakinetid nucleus and possibly plays a part in spindle formation.
5. Twenty-one chromosomes are present at heterotypic metaphase. These consist of nineteen bivalents and two unpaired univalents of larger size. The univalents remain undivided and twenty chromosomes pass to each daughter nucleus.
6. The homotypic chromosomes are of extremely small size. The divisions show no irregularities and cytokinesis appears to take place by furrowing.

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## EXPLANATION OF PLATES I AND II.

Illustrating Dr. Joan Latter's paper on the Meiotic Divisions in the Pollen Mother-cells of *Malva sylvestris*.

### PLATE I.

All figures were drawn with a camera lucida. With the exception of those mentioned below, all were drawn under a 2 mm. imm. Zeiss N.A. 1.4 with comp. oc. 30 ×. Magnification = 4,300.

Figs. 1, 8, 13, 14, 15, 18, 19 were drawn under a 4.2 mm. Zeiss with comp. oc. 15 ×. Magnification = 1,000.

Figs. 2 and 4 were drawn under a 2 mm. imm. Zeiss N.A. 1.4 with comp. oc. 20 ×. Magnification = 2,950.

The drawings have been reproduced without reduction.

*Abbreviations*: C. = Carnoy fixation. A.B. = Allen's Bouin fixation. C.A. = Chrome acetic fixation.

Fig. 1. Pollen mother-cell with resting nucleus. Dark aggregations of granules are present in the cytoplasm. C.

Fig. 2. The granular reticulum is gathered at one side of the nucleus to form the synizetic knot. Nucleolar material is extruded into this granular mass. A.B.

Figs. 3 and 4. The spireme is connected to dark-staining nucleolar bodies. The nucleoli are vacuolate and show budding. C.

Fig. 5. Parallel association of separate strands of spireme after loosening from synizesis. All the spireme is not figured. C.

Fig. 6. Tangential view of nucleus showing the formation of bivalent threads by twisting of the parallel strands about one another. C.

Fig. 7. Early diakinesis. Bivalents are assuming a peripheral position, while a faintly staining reticulum still surrounds the nucleolus. C.

Fig. 8. The first appearance of the perinuclear zone around a diakinetid nucleus. The zone merges into the cytoplasm. C.

### PLATE II.

Fig. 9. Late diakinesis. All the bivalent chromosomes are peripheral: two were further displaced in the cytoplasm and are not figured. The total width of the perinuclear zone is not shown. C.

Fig. 10. Heterotypic metaphase plate with twenty-one chromosomes. C.

Fig. 11. The onset of anaphase. The bivalents have divided, while two others show the start of the anaphase split. C.

Fig. 12. Polar view of early anaphase. Thirty-eight chromosomes are still associated as nineteen pairs, and two larger unpaired univalents are present. C.

Fig. 13. The heterotypic spindle is completely surrounded by the deeply stained perinuclear zone. C.

Fig. 14. Interkinesis with formation of perinuclear zones about the daughter nuclei. C.

Fig. 15. Perinuclear zones surrounding the homotypic nuclei. C.

Fig. 16. Polar view of homotypic anaphase group with twenty chromosomes. A.B.

Fig. 17. Side view of homotypic spindle showing twenty chromosomes in each group at the poles.

Fig. 18. Perinuclear zone material occupying most of the protoplast area at the reconstitution of the daughter nuclei. A.B.

Fig. 19. Aggregation of the granular matter into a perinuclear zone round each tetrad nucleus. C.A.

# A Contribution to the Physiology and Anatomy of Tracheae with Special Reference to Fruit Trees.

## II. Water Conductivity in Higher Plants and its Relation to Tracheae.

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With one Figure in the Text.

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### INTRODUCTION.

IN the previous paper on this subject the writer brought forward some data which suggested that in cross-section a unit area of the lumina of the tracheae of prune wood is 49 per cent. more efficient in its capacity to conduct water than a similar unit area of the lumina of tracheae of apple wood. In presenting this view an assumption was made that the

liquids passing through the tracheae of various plants have the same viscosity. Various external conditions affecting transpiration (other than the transpiration power of the type of the leaf itself), as well as the pressure under which these liquids were conducted, were also granted to be similar for both kinds of trees studied. In this paper it will be attempted to show experimentally that, whereas a unit area of tracheae of prune wood is 49 per cent. more efficient in its capacity to conduct water than apple, some other factor or factors are more efficient in apple than prune, which compensate for this efficiency in favour of the prune wood.

It is hoped that the elucidation of such factors might be of some help in forming a true picture of water conductivity.

#### HISTORICAL REVIEW AND COMMENTS.

Farmer (12, 13) studied the quantitative differences in the water conductivity in the evergreens and deciduous plants, and found that the specific conductivity of evergreens is relatively low and of the deciduous trees relatively higher. He attributed this difference to be due to the narrower and shorter water-conducting elements possessed by evergreens.

Holmes (20, 21) made observations on the water-conducting elements of hazel and ash wood, with reference to their water conductivity. She found lower specific conductivity in ash wood while higher in hazel wood. This difference in conduction in favour of hazel wood she considered to be due to the greater number of water-conducting elements per square millimetre in that wood.

Rivett (30) studied the anatomy of *Rhododendron ponticum* L. and of *Ilex aquifolium* L. in reference to their specific conductivity. He compared the relative cross-section area of these vessels as well as those used by Holmes, and concluded from that study that it was the length of the vessels, rather than the balance between size and number of the vessels, which was the important factor in water conductivity.

Clements and Loftfield (6) have studied the length of the tracheae and the specific and absolute conductivity of some herbaceous plants by means of a modification of Farmer's method. They found an increase in both specific and absolute conductivity from the base to the top. They placed emphasis on their statement that in many cases the length of the stem had practically no influence on the water conductivity.

On summarizing the papers reviewed so far, it is very apparent that the importance of size, length, and number of the wood elements only has been stressed in water conductivity. Yet the velocity ratio of liquids passing through a space may be so different that it could compensate for the dimensions, which have been emphasized by these authors.

There is no doubt that area, number, and length of the wood elements have a profound influence on the conduction, as these authors have so ably shown, and as was suggested by the writer in the previous paper. But these constitute just one of the phases upon which water conductivity depends, as pointed out by Ewart (11). He says: 'In vessels filled with water, the resistance to flow depends upon the rate of flow, the square of the radius of the tube, the length of the vessel and the viscosity of water.' It is true that the velocity of a gas or of a liquid diffusing or forcing through a cylinder is influenced in part by the dimensions of the cylinder, as demonstrated by Brown and Escombe (3). But it also has been proven experimentally that the velocity of liquids depends upon their viscosity. In fact, the term viscosity, as first described by Poiseuille (19), and the various methods by which the viscosity of liquids is measured depend on the velocity of liquids passing through capillary tubes of one sort or another, commonly known as a viscosimeter. Michaelis (26) describes one of these methods in his book. Recently Inamdar and Shrivastava (22) have studied the relationship between the specific conductivity and the structure of the wood in some tropical plants. They pointed out that, in general, the diameter of the vessels varied in the same direction as the specific conductivity. However, they also emphasized the fact that the diameter of the vessel was not the only contributing factor towards variations in the specific conductivity, as they illustrated by the fact that no quantitative proportionality was maintained between the diameter of the vessels on the one hand and specific conductivity on the other. They thought that the chief cause for variation in the specific conductivity was the resistance offered by the wood.

It seems to me that variations in the specific conductivity, as recorded by Inamdar and Shrivastava (22) at various times of the year, may be due to the variations in velocity of liquid passing through the vessels. This velocity may be affected by changes in temperature, which is true of liquids in general, as recorded by Knowlton (23) and Worthington (33). Most liquids undergo a progressive decrease in viscosity as the temperature is raised, as described by Heilbrun (18). As the viscosity of a liquid decreases, due to increase in temperature, as the data of Hodgman and Lange (19) show, its velocity increases also, assuming that the pressure and other conditions are the same. This view can be supported by any text-book on physics. For instance Spinney (31) says: 'It is thought that molecules of a hot body are in the state of rapid vibration, and that the hotter the body is the more rapid is this vibratory motion of its molecular parts.' Thus it is not surprising that Inamdar found maximum water conductivity before leaf-fall in the plants he studied.

## MATERIAL AND METHODS.

A. *Extraction of sap.*

Since Delicious apple and French prune shoots were found to be the most promising material, as indicated by the previous study, about 100 apparently healthy shoots, approximately 80 cm. long, were selected on June 11, 1928, from each of these kind of trees. The leaves were immediately stripped off, shoots cut into small pieces, and sap from the lumina of tracheae was extracted by the 'gas displacement method', following all the details as recorded by Bennett, Anderssen, and Milad (2).

There are other methods of extracting sap, namely, the centrifugal method, as discovered by Dixon and Atkins (8), and the compressed-air method, as described by Dixon and Ball (9). The limitations of both of these methods with reference to their application in the extraction of sap in woody plants has been discussed by Chibnall and Grover (5) and by Bennett, Anderssen, and Milad (2). The latter authors have also pointed out the various advantages of sap extraction by the 'gas displacement method' in woody plants. An average shoot 80 cm. in length yielded 13–16 mm. of uniform sap by this method. The sap so obtained was kept separately for apple and prune tracheae and sealed until used.

B. *The determination of the density of the sap.*

First, the density of the sap was determined in duplicate at 20° C., and at the same atmospheric pressure, by Westphal Balance, following the direction of Gray (16).

C. *The determination of the viscosity of the saps.*

The relative viscosity of the saps was determined in duplicate by Ostwald's viscosimeter, according to the directions laid down by Gray (15). A viscosity tube was placed in the oil (instead of water) thermostat, with a constant temperature of 25° C. Each time a viscosity measurement was made in an absolutely clean and dry tube. Air-free distilled water was used for standard. 10 c.c. volume was used in all measurements. The coefficient of viscosity for the saps used was calculated by the following formula :

$$\frac{n}{n'} = \frac{t \times d}{t' \times d'}.$$

$n$  and  $n'$  are coefficients of viscosity for the sap and water respectively ;  $t$  and  $t'$  are time in seconds required for 10 c.c. of sap and water respectively to flow through capillary tubes under the standard conditions of the experiment.

$d$  and  $d'$  are density of the sap and water respectively.

The value of  $n'$  for water at 25° C. was taken from the experiments rather than  $8.926 \times 10^{-3}$  (accepted figures).

Figures for density, viscosity, as well as for the later experiments to be reported herein, were calculated according to the procedure of Daniels (7).

*D. Quantitative determination of the chemical composition of the saps.*

The figures obtained from the above experiments showed that there was a difference in viscosity of saps obtained from the apple and prune shoots. Since it is held that differences in viscosity have an effect on the velocity of liquids, it is of interest to find the velocity of these saps under the same pressure, because this may show the difference in the rate of water conductivity in their respective shoots when all other conditions, even the acceleration effect due to gravity, are similar.

Since the saps obtained from the shoots were not sufficient for the determination of velocity, the only alternative was to make up similar saps. To do this a chemical analysis of the composition of the original sap was made. Later, similar saps of the composition determined by the analysis were prepared in the laboratory.

In the chemical analysis the amount of sugars was determined by picric acid reduction, method of Thomas and Dutcher (32).

Picric acid was purified, as suggested by Folin and Doisy (14). A more dilute solution of mercuric nitrate was used as clarifying agent than the one used by Benedict and Osterburg (1).

Mercuric nitrate was used because Browne (4) and others have regarded the use of basic lead acetate as unsatisfactory for the clarification of sugar extracts.

No effort was made to segregate the various kinds of proteins present. Analysis for the determination of total proteins by the Arnold-Gunning modification of the Kjeldahl method was carried out, as outlined by Mathews (25). The total ash content was determined very accurately by burning organic matter in the electric muffle, following the method of Hawk (17).

No attempt was made to determine various inorganic constituents, although their presence or absence was detected by microchemical analysis from Molisch's (27) directions.

All chemical analysis of sugars, proteins, and ash (Table I) were run in triplicates, and a check within 0.5 per cent. was considered accurate enough for this study. The results were expressed in parts per million. All calculations were compiled after Hamilton and Simpson's method (16).

The data obtained from the above analysis were used in the preparation of a fluid similar to the sap obtained from the lumina of apple and prune shoots. Various constituents were calculated in p.p.m. per litre, mixed, and a corresponding sap was prepared in such a manner that the chemical composition, density, and viscosity of the sap was about the same as was found in the original saps of apple and prune shoots. It must be admitted

that there may be several objections to the preparation of saps by this method. For instance, although the total ash in these solutions was present in the same amount as in the original, yet the percentage of various elements in the ash, calculated as oxides, may or may not have been the same as found in the extracted sap.

These percentages of the various elements of the total ash were assumed to be the following:  $K_2O$  14.40;  $CaO$  60.20;  $MgO$  4.50;  $Fe_2O_3$  2.30;  $P_2O_5$  2.70;  $SO_3$  3.50; and  $SiO_2$  10.0—as tabulated by Palladin (28) for the percentage of the various elements in the dry weight of ash of *Fagus* wood. It seems to me that in such an attack as this a procedure of this nature is inevitable. A possible error, due to this factor, might be so small that it can fall within the limits allowed for experimental error.

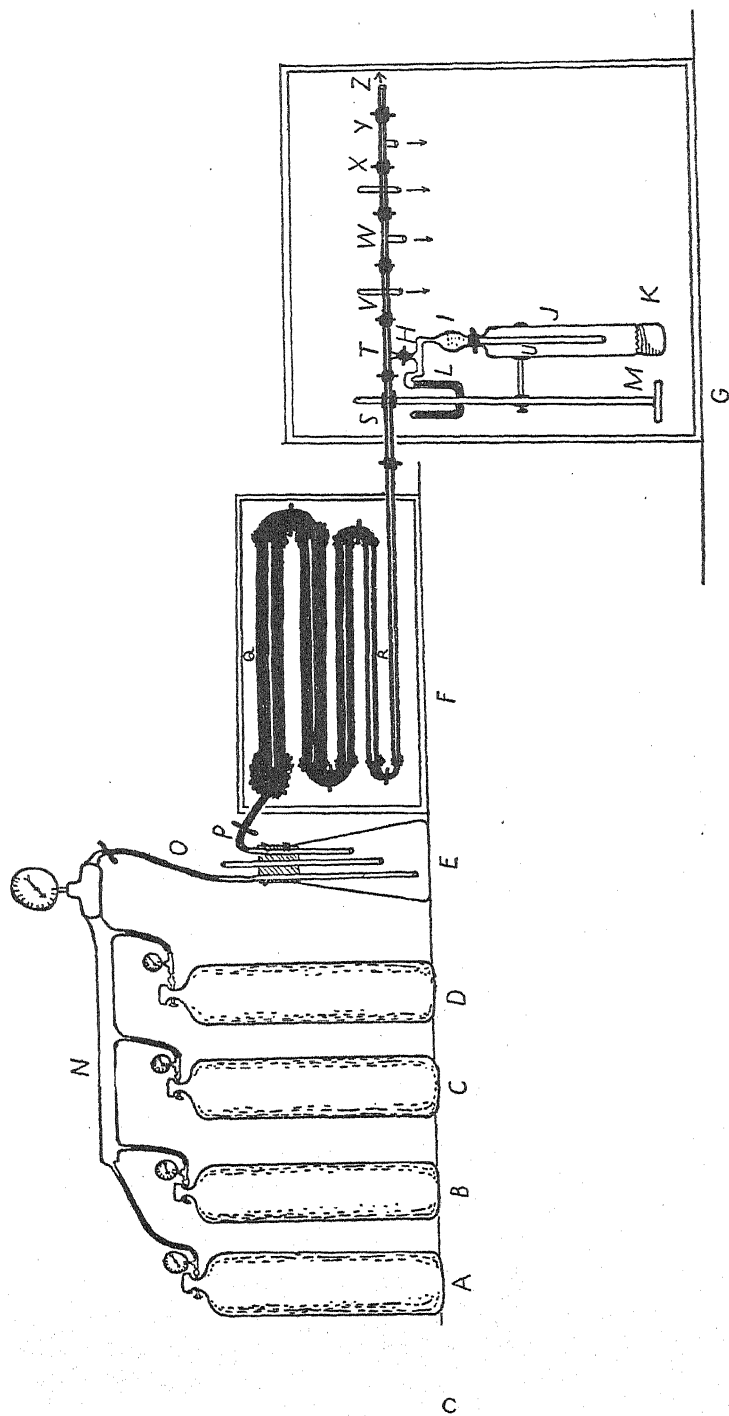
#### *E. The determination of the velocity of the saps.*

From the previous experiments undertaken in this study, it was found that the liquids extracted from the lumina of the tracheae of apple and prune shoots varied in density, viscosity, and chemical constituents. It is to be expected that the velocity of these liquids may vary also. It is possible that the differences in the water conductivity of these woods may in part be due to the differences of the velocity of the liquids themselves. In order to determine the relative variation due to the liquids only it is essential that all other factors of variation be eliminated.

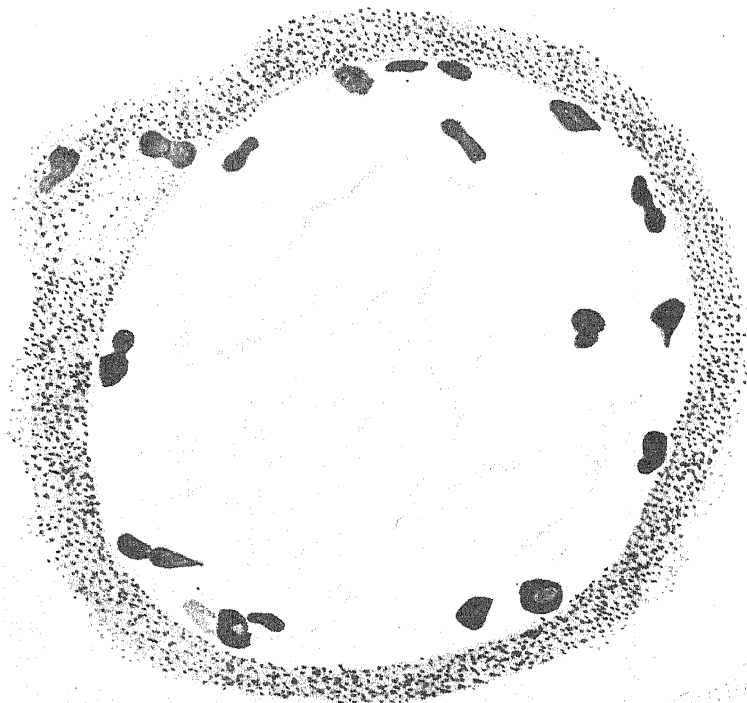
The apparatus as shown in Text-fig. 1 was set up for this study. Four small cylinders (A, B, C, and D) full of oxygen, with pressure meters at the outlets, were secured. They were connected with a brass pipe (N) by means of strong rubber tubings; each one of these tubings could be closed when desired. At the head of the brass cylinder another pressure meter was installed. This meter recorded the total pressure under which oxygen passed out. At the outlet of the cylinder another rubber tubing (O) was fitted tightly, sealed with wax, and wrapped with steel wire. Below this sealing a strong screw clip was attached for closing, opening, or adjusting the flow of the oxygen. The use of four small cylinders instead of one large one was considered expedient, because their delivery insured a more gradual and uniform pressure of gas. These conditions seemed to be safer and also more convenient for starting a minimum flow of oxygen. Furthermore, the use of the four small cylinders produced an apparatus which was much more easily and accurately controlled.

A thick pyrex glass flask (E), with a capacity of 500 c.c., was sealed by a three-holed rubber stopper. This stopper was made air-tight by means of wax and steel wires. These holes were fitted with three thick glass tubings. The outer end of one of these tubings was fitted to the rubber tubing (O), through which oxygen was forced into flask (E). At the upper end of the

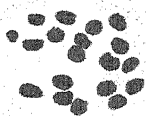




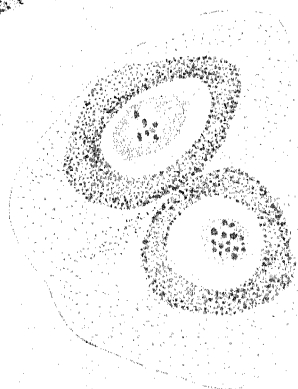
Apparatus for measuring the water conductivity of woody shoots. Gas tanks A, B, C, D are connected with brass cylinder N by heavy rubber tubings. E is 500 c.c. pyrex flask with outlet. Q to R heavy glass tubes in box F. Chamber G with six outlets—I reservoir, U capillary tube, L monometer, J protecting glass cylinder, K graduated cylinder supported by cork plate. Pyrex glass was used in all cases; rubber tubing used was heavy. For details see pp. 16-19.



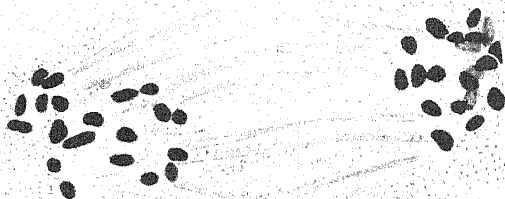
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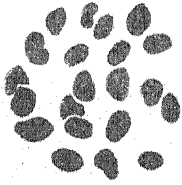
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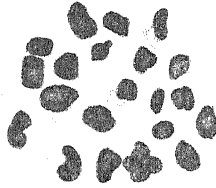
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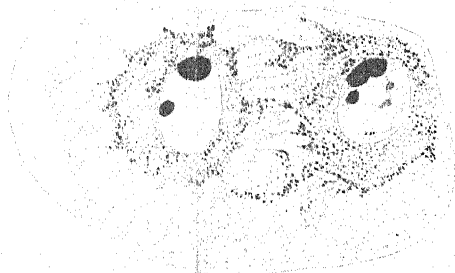
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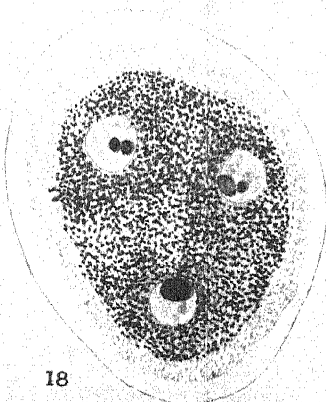
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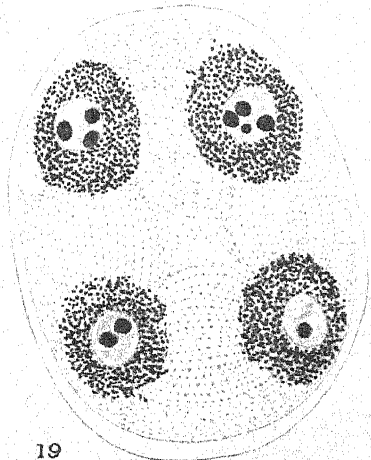
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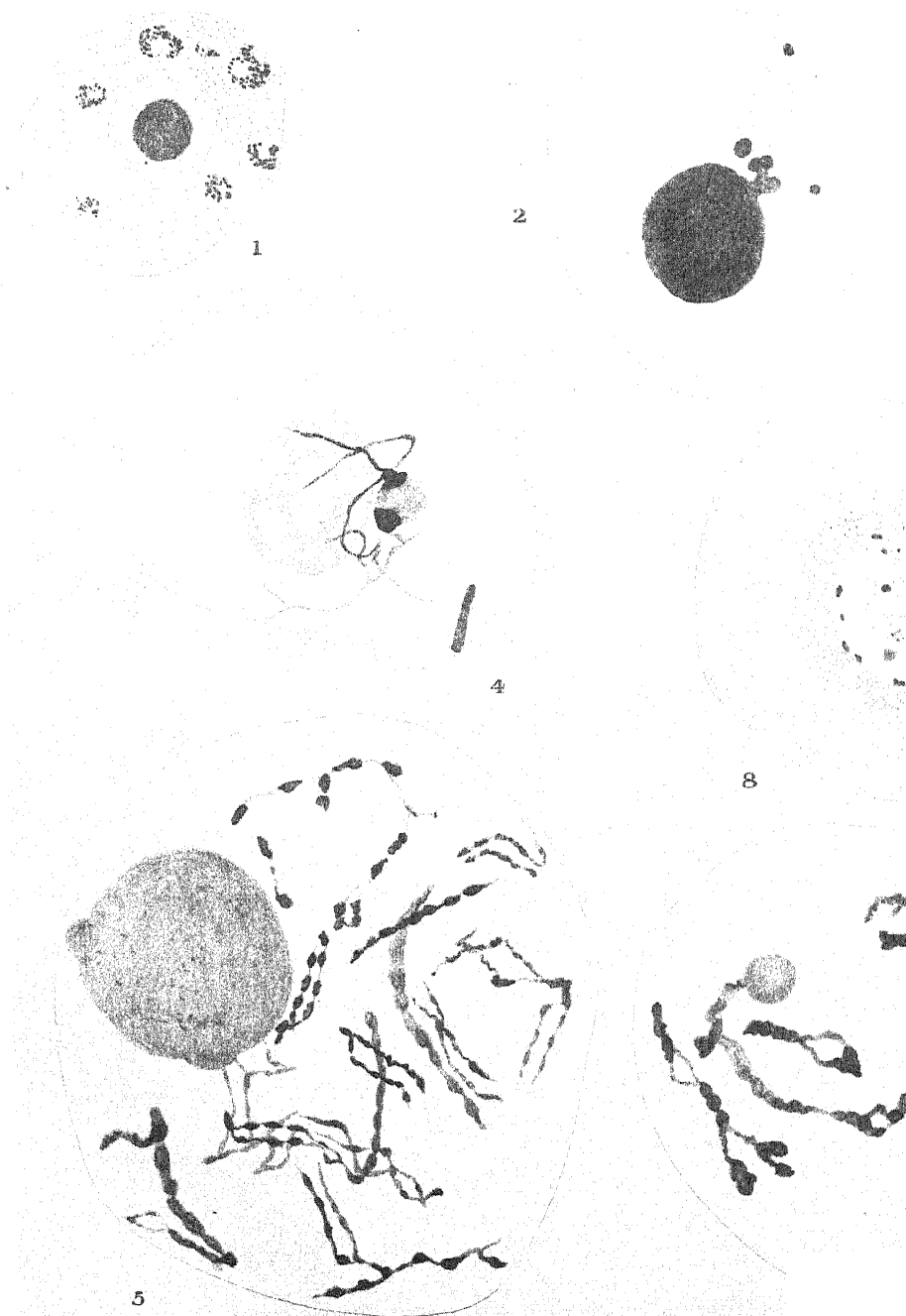
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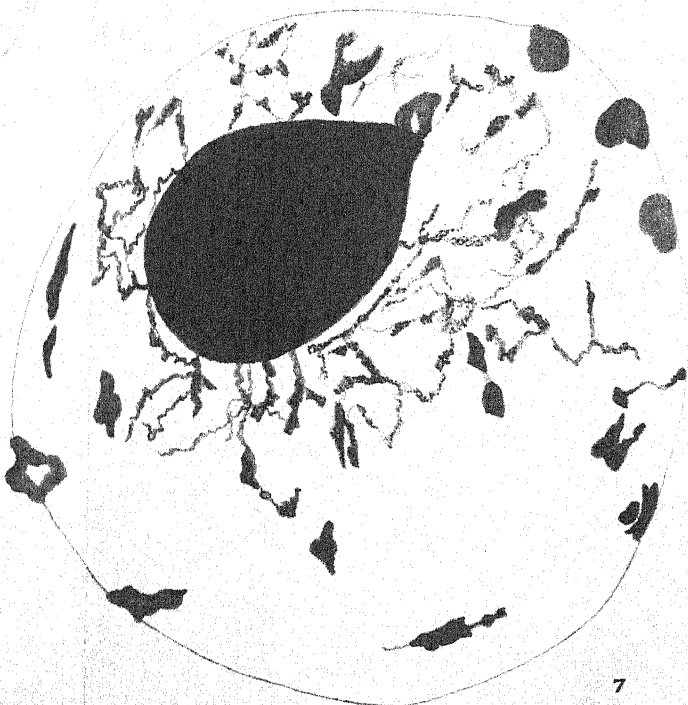


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middle tube a soft piece of rubber tubing was attached and a screw clip placed at the top. This clip when opened served as a safety valve. At the head of the third glass tube rubber tubing (P) was connected, in such a manner as tubing (O), to allow the oxygen to be forced toward the apparatus (F). Flask (E) acted as a safety device, permitting the adjustment of excessive pressure without causing a fluctuation in the main pressure of the cylinders. It was wrapped with a netted wire sheet as a further protection in case of accident. The other end of the rubber tubing (P) was connected with a heavy pyrex glass tubing (Q), which was 2 cm. in diameter with a half-centimetre bore. Tubing (P) was fixed with a screw clip to control the pressure of the oxygen passing toward (Q). Below (Q) four more glass tubings were constructed in the shape of a coil. They were connected by rubber tubings at the end points, which were sealed with paraffin, then with wax, and finally with wires. These tubings decreased gradually from (Q) to (R) in their diameters, as well as in the diameters of the bores. Such an arrangement seemed necessary for better control of the gas, safety, and the perfect connexion of tubing (R) with the rest of the apparatus. The diameter of the tube (R) was the same throughout, and was the same as that of tube (S). All of the rubber tubings between (Q) and (R) were fixed with screw clips. This whole system, (Q) to (R), was enclosed in a wooden box with borings for rubber tubing (P) to go in and the glass tubing (R) to pass out. Two more borings were made on the front side of the box. These permitted the operator to watch the contents of the box when necessary. The box served as a protection to the tubings as well as to the operator.

Chamber (G) was constructed in such a manner as to permit one of its sides to be opened and closed by means of hooks. Within this chamber (G) was placed an iron stand (M) with two clamps, one to hold the pyrex glass cylinder (J) and the other to hold the glass tube (S) in place. Glass tube (S), which was allowed to pass from the boring made in chamber (G), was connected with glass tubing (R) by means of rubber tubing. A screw clamp and a safety device were fixed at the junction of these tubings. At the other end of (S) a T-shaped glass tubing (T) was connected by means of a rubber tubing. Tube (T) was connected with another T-shaped tube (H). On one end of the tube (H) a monometer (L) was sealed as shown in the plate. At the other end of (H) a graduated reservoir (I) with a capacity of 50 c.c. was connected. At the lower end of the reservoir (I) a capillary tube (U) of 0.5 mm. opening was connected by rubber tubing. This rubber tubing could be opened by means of a screw clip. The capillary tube (U) was enclosed in a pyrex cylinder (J), the lower end of which was screwed with graduated cylinder (K). Cylinder (K) was supported by a thick cork plate which, when taken off, permitted screw connectings (J) and (K) to be unscrewed and the contents of (K) to be emptied. The cork plate is not

shown in the diagram. The purpose of this cylinder (K) was to catch the liquid, which passed from reservoir (I) through the capillary tube (U).

On the front side of the chamber (G) facing the operator, just opposite to cylinder (K), a glass was so inserted in the wall of the chamber as to permit observation of the volume of the liquid collected in the cylinder. This eliminated the disturbance of cylinder (K) at the time of reading. It also permitted more satisfactory measurement of the volume of the liquid present, since (K) was protected by (J) as well. This arrangement eliminated the chance of evaporation, which might have caused an error in the experiment. It also avoided the danger of the spilling of the liquid. The cylinder (J) was 20 cm. long. The capillary tube (U) was replaced by many other tubes of different dimensions. Afterwards the same place was occupied by apple and prune shoots of various diameters and lengths. There were five more set-ups like the one described above. They were connected with tubing (V, W, X, Y, Z). The arrows in the drawing show their positions. This arrangement permitted reading of the conductivity of half-a-dozen capillary tubes or shoots at the same time. All glass tubing from (R) to (Z) was of the same size and thickness.

Chamber (G) served for protection of the apparatus and the operator in much the same way as did box (F). In an experiment of this sort, in which a considerable pressure is to be exerted on the glass instruments, it is always safe to take extra precaution. At the same time conditions for greater accuracy are possible. It may be mentioned also that all connexions were tested for leakage so that the apparatus, when completed, was tight. In all cases heavy pyrex glass was used, because it was safe, and at the same time easy to handle. The rubber tubing used also was heavy, except in outlets or safety valves.

The capillary tubes were made of a heavy pyrex glass with perforations of 0.07 mm. to 1.2 mm. in diameter. Their length varied from 9 to 15 cm. The accuracy of the diameter of these perforations was checked by means of a binocular. The lengths of the tubes were measured under a lens by means of a draftsman's calibrated ruler. This range of the diameters of the perforations in the tubes was chosen because it was learned from the previous study that the maximum and the minimum diameters of the lumina in apple and prune wood fell within these ranges. The selection of the lengths (between 9 and 15 cm.) of the capillary tubes was made for a similar reason as the portion of shoot worked with was 9 to 15 cm. long. It was learned in the study of the distribution of tracheae in these shoots that half-a-dozen tracheae appeared within 6 cm. after the appearance of the first open trachea, and as it was desired to measure the flow of half-a-dozen tracheae a portion of the shoot from 9 to 15 cm. in length was measured off and cut from the point where the first open trachea was visible. When the apple and prune shoots were substituted for the

capillary tubes the conductivity of the shoot was measured only through six individual tracheae, whose lengths and diameters were determined individually by the methods described in the previous paper. First the liquid with the chemical composition, density, and viscosity of the apple sap was forced through the capillary tubes of various sizes and lengths prepared as described above. The liquid prepared, comparable to prune sap, was next run through these tubes. Then these two liquids were forced through apple and prune shoots. In all cases liquids were forced through the tubes or the shoots, in duplicate, for 10 minutes, at a pressure of  $1\frac{1}{2}$  atmospheres. It was assumed that the pressure exerted [at any point upon the mass of a liquid was transmitted undiminished in all directions according to Pascal's law, as stated by Richtmyer (29).

It was realized that in a few cases the diameters of the perforations of the tubes and the diameters of the lumina of the tracheae in apple and prune wood might not be the same. Yet it was deemed essential that the diameters of the opening of the tubes and of the tracheae be the same if any results were to be deduced from this study. In order to do that the liquids through the minimum up to the maximum diameter and lengths were forced under the standard conditions described. The volume of the liquid obtained was taken as a measure of the conductivity of the individual tube of that specific diameter and length. When such a reading for all the tubes was completed the data was reduced to one variable only, namely, the diameter. In other words, the values were all reduced to a uniform length of 15 cm. but different diameters ranging from 0.07 to 1.2 mm. The same process was repeated for both liquids. The data obtained from the above was plotted on two different graph sheets in a system of fixed rectilinear co-ordinate axes.

The abscissa represented the diameter of the perforations while the ordinate represented the volume transmitted under standard conditions. The points on the charts were located and a curve was plotted which gave the conductivity values at any point between these ranges. In the preparation of these interpolation charts LaCroix and Ragot (24) were consulted. It may be argued by some that the use of an interpolation chart is objectionable. Probably this is true of extrapolation charts, since they involve the calculation, from the values of a function known within a certain interval, of values of its argument, lying outside that interval. An interpolation chart, on the other hand, is the method or operation of finding approximately from given terms of a series, as of numbers or observations, other intermediate terms, in conformity with the rule or law given or assumed, of the series.

Even in physical science the use of interpolation charts is permissible because the approximate accuracy of the values of a function is available by experimentation. Thus it seems to me that such an objection



cannot stand against the experimental evidence and figures. For this study the use of an interpolation chart seems to be accurate enough.

It has been stated by some authors (10) that the inner surface of the cells may be irregular and lignification of the walls of tracheae be uneven. If the walls of the apple and prune tracheae possess these differences also one would expect the resistance afforded by these walls on the conductivity might be variable. By measuring the volume run through a tube with a glass wall and comparing with that run through comparable tracheae, figured on the basis of standard diameter, length, and other conditions in the apple and prune shoots, we can figure out the resistance due to the nature (mechanical or chemical) of the wall. When these figures for the resistance due to the wall variations are subtracted from the total figures of the conductivity (per unit length and diameter of tracheae under standard conditions) the remaining net volume of the liquids transmitted indicates the difference due to the liquids themselves (density, viscosity, and chemical compositions). The diameter and area of tracheae were determined by the method noted in the previous study.

In this experiment, the acceleration effect due to gravity has been assumed to be uniform. The heat produced by 1 or  $\frac{1}{2}$  atmospheric pressure has also been assumed to be the same in all cases. Thus no correction has been made for these two factors. The data and the results will be discussed elsewhere.

Velocity of the rate of flow of liquids is generally found by this method, namely:

$$\text{The rate of flow} = \frac{\text{Quantity of the liquid}}{\text{Time it required to pass}} = \frac{Q}{t}$$

Quantity ( $Q$ ) in a cylinder (as the perforation of the capillary tube or tracheae) =  $\pi r^2 \times \text{length}$ . Time ( $t$ ) is the same in all cases. Therefore in determination of the rate of flow in two tubes or tracheae of different dimensions, the ratio would be:

$$\frac{Q^1}{Q^2} = \frac{\pi r_1^2 l_1}{\pi r_2^2 l_2} = \frac{r_1^2 l_1}{r_2^2 l_2}$$

divided by the common time ( $t$ ). By this method the rate of flow for all the capillary tubes and the tracheae was calculated. It was assumed in order that water may flow in a pipe there must be difference in pressure from point to point along the pipe, i. e., the pressure decreasing in the direction of the flow, as is commonly demonstrated in experiments of the fall of potential. Since resistance and flow both in hydro-dynamics and electrokinetics obey the same laws (31) the resistance was determined by

the formula, namely:  $R = \propto \frac{L}{q}$  where  $R$  is resistance.  $L$  is length of the

lumina or perforation, and  $q$  is area of the cross-section ( $\pi r^2$ ). When the total resistance of all the tracheae in one shoot was to be determined the laws of the resistance of conductors in parallel and series were applied, namely, 'The resistance of a branched circuit is equal to the reciprocal of the sum of the reciprocals of the resistances of the branches', which, when algebraically expressed, is as follows:

$$R = \frac{1}{\frac{1}{r_1} + \frac{1}{r_2} + \frac{1}{r_3} \dots}$$

in which  $R$  = total resistance;  $r_1, r_2 \dots$  are the resistance of the individual tracheae (32). The resistance of an individual tracheae is determined by its flow, or by subtracting the flows of other tracheae except the one to be determined.

#### DISCUSSION AND PRESENTATION OF DATA.

##### A. *Physical properties of the sap.*

1. *Density.* Table I column 3 shows the density of the saps extracted from the lumina of apple and prune wood. It will be noted that prune sap had more density than apple sap at 20°C. In other words, prune sap was found to be heavier per unit volume than apple sap. Even when both kinds of sap were examined by the naked eye, it was observed that prune sap might have more solid bodies than the apple sap. Density of prune sap was 0.26 per cent. higher than density of distilled water, while density of apple sap was 0.15 per cent. higher than distilled water. The percentage difference between apple and prune sap was 0.11 per cent.

2. *Viscosity.* Table I column 4 shows the viscosity of the two kinds of saps. It can be noted from those figures that the coefficient of viscosity in C.G.S. units at 25°C. is higher in prune sap than in apple sap. In other words, prune sap was more viscous than apple sap. No parallel correlation has been found between the density and viscosity of the two saps studied. Whereas there was 0.16 per cent. difference in density, there was 2.03 per cent. difference in viscosity of apple and prune saps, although in general the tendency of both the physical properties of sap, namely, viscosity and density, were higher in prune than in apple. These physical properties seem to show a decided relationship with the chemical determination.

##### B. *Chemical study of the saps.* (Table I, columns V, VI, and VII.)

1. *Sugars.* The amount of sugars present (determined as glucose) was higher in prune sap than in apple sap when figured as p.p.m. When these figures were changed to p.p. 100, apple sap showed 1.120 while prune sap showed 1.319. The per cent. difference between apple and prune was 0.199.

TABLE I.

Data showing some physical and chemical properties of the sap from the lumina of the tracheae in apple and prune wood. These properties were determined quantitatively by the methods described in this paper.

In all cases an average of duplicate analyses is reported.

Serial No.	Kind of shoot.	Properties of sap.				
		Physical.		Chemical p.p.m.		
		Density at 20° C.	Coefficient of viscosity C.G.S. units at 25° C.	Sugars.	Proteins	Ash.
I.	II.	III.	IV.	V.	VI.	VII.
1.	Delicious apple	1.0003	0.00904	11,201	22	600
2.	French prune	1.0019	0.00923	13,199	27	709
3.	Redistilled water	0.9988	0.00891	—	—	—

2. *Proteins.* The quantity of total proteins expressed in p.p.m. was greater in prune sap than in apple sap. If the figures for sugar and protein variations between the two kinds of sap be changed into percentages, it would be found that differences in percentage were variable, i. e. in sugar these variations were 0.199, in proteins 0.0005. In other words, there was no direct correlation between the variations of these two chemical constituents of the saps.

3. *Ash.* The total ash figured as p.p.m. was also greater in prune sap than in apple sap of these shoots. When p.p.m. were changed to p.p. 100 it was found that the figure for apple sap was 0.060, while that for prune sap was 0.071. It was surprising that the extracted sap from the shoots of apple and prune trees grown in the same soil, under the same conditions, was different in these chemical properties.

C. *Determination of the resistance offered to the flow of the saps due to the nature of the walls.*

Table II figures in series I show that the volume of prune sap passed through capillary tube, apple tracheae, and prune tracheae was 7 c.c., 6.3 c.c. and 5.5 c.c. respectively. This passed through the perforations under standard conditions (pressure  $\frac{1}{2}$  atmosphere, length 15 cm.). If the capillary tube walls are to be taken as walls which exert standard resistance to the flow of the liquid, then the difference represented by columns IV and V would be due to the resistance of the walls of apple and prune tracheae. Columns VI and VIII show this difference due to the nature of the walls of the two kinds of tracheae. Absolute difference of conductivity due to

the resistance offered by the walls, under standard conditions, was 0.7 c.c. in case of apple and 1.5 c.c. in case of prune. Columns VII and IX show these values in percentages. By subtracting these percentages it was found that sap of the prune when passed through apple and prune tracheae gave different results. The walls of prune tracheae offered 11.4 per cent. more resistance to water conduction than walls of the apple tracheae. This is evident from column X. When apple sap was substituted for prune sap, it was found (columns III and V) that the capillary tube yielded 7.95 c.c., apple 7.25 c.c., and prune 6.45 c.c. Thus it was found that for apple sap the percentages of total resistance which is due to walls was 8.8 for apple and 18.9 for prune (columns VII and IX). Subtracting these figures, it was found that for apple sap prune walls gave 10.1 per cent. more resistance than did the apple wall. By taking an average of both these differences the figure derived was 10.75 per cent. In other words, the walls of the tracheae of prune wood offered 10.75 per cent. more resistance to the flow of sap than the walls of apple tracheae did.

TABLE II.

Data showing the resistance offered to the conduction of water in apple and prune tracheae due to differences in the nature of their walls. (In all cases the data are reduced to the standard conditions, namely, length 15 cm., diameter 0.5 mm., pressure  $\frac{1}{2}$  atmosphere, temperature 21° C., and time 10 minutes.)

Serial No.	Kinds of saps used.	Volume in c.c. collected at the standard conditions from:—			Resistance due to the nature of wall in the tracheae of:—				% differences between resistance offered by the wall of tracheae of apple and prune tracheae.
		Capillary tubes.	Apple tracheae.	Prune tracheae.	Apple.		Prune.		
					Absolute.	%.	Absolute.	%.	
I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.
1.	Prune (French)	7.0	6.3	5.5	0.7	10.0	1.5	21.4	11.4
2.	Delicious apple	7.95	7.25	6.45	0.7	8.8	1.5	18.9	10.1
Average									10.75

D. *Determination of the resistance offered to the flow of the sap in the lumina of apple and prune wood due to their saps.*

Data in Table III show the figures of the resistance due to the liquids themselves. By referring to columns III and IV, it will be seen that 7.95 c.c.

of apple sap passed through a unit of capillary tube, while only 7 c.c. of prune sap passed under the same conditions.

TABLE III.

Data showing the resistance in the lumina of apple and prune tracheae due to the saps (apple and prune)—c.c. of saps collected from borings per unit area with the conditions the same as noted in Table II.

Series No.	Kind of perforations used.	Volume collected in c.c. (per unit length and cross-section) with the sap extracted from the tracheae of:—		Resistance offered to the passage of liquid due to the saps.	
		Apple.	Prune.	Absolute.	Percentage.
I.	II.	III.	IV.	V.	VI.
1.	Capillary tube	7.95	7.0	0.95	13.6
2.	Apple	7.25	6.45	0.80	15.0
3.	Prune	6.45	5.55	0.90	16.3
Average of apple and prune				0.85	15.65

Series 2 and 3 show the same sort of figures for a unit length and diameter of apple and prune tracheae.

Columns V and VI show the absolute and percentage of resistance in capillary tubes, apple and prune tracheae, due to apple and prune saps. It will be noted that 13.6 per cent. resistance was registered in capillary tube, 15.00 per cent. in a unit length and diameter of apple tracheae, and 16.3 per cent. in prune tracheae. In other words, these figures represented the greater resistance offered by the prune sap than the apple sap, whether in the capillary tube, in apple tracheae or in prune tracheae, when either of these saps were forced through. The average of these percentages was 15.65 per cent.

Thus it seems from the data presented in Tables II and III that the tracheae of prune offers 10.75 per cent. more wall resistance than the tracheae of apple; and 15.65 per cent. more resistance in prune than apple due to the nature of the sap, making a total resistance of (10.75 + 15.65) 26.40 per cent. due to these two factors. The data in the last paper indicated that a unit area of prune was 49 per cent. more efficient in its water conductivity than a similar unit of apple area. If we deduct from this percentage (49) in favour of prune, that (26.40) in favour of apple, the figures will be 22.6 in favour of prune. In other words, the efficiency of conduction in favour of prune due to the factors (shown in the previous papers) of tracheae in prune wood are more or less compensated for by the nature of the walls and the fluid in apple tracheae. Of course, there is still a difference of (49.0 - 26.4) 22.6 per cent. in favour of prune in

its efficiency to conduct water per unit area. There may be some other factors responsible for this, of which the writer is not aware ; but reviewing the literature no other possibility suggested itself.

It may be that a part of this difference of 22.6 per cent. in favour of prune can be attributed to experimental error, since the method of attack demands many operations of one sort or another. Even this 22.6 per cent. is not within the range of the experimental error. This is particularly reasonable when we consider the preparation of artificial saps.

In the preparation of these saps it was pointed out that the figures for the percentage of ash used to make up the solution were taken from Palladin (28). This was necessary, because the total ash yielded by these saps was so small that it did not permit quantitative determination of individual inorganic ingredients of the total ash. It may be that this percentage of ash was not what was found in these saps. Thus it seems that the error due to this factor may account for a part of the difference of 22.6 per cent., which is beyond the range of experimental error.

From this study it is evident that in some ways prune tracheae are more efficient than those of Delicious apple. In the total capacity to conduct water, however, they reach a point of equilibrium, or near a point of equilibrium. Our general concept of tracheae and their functions would be nearer the truth if we also considered the nature of the sap itself, as this study has attempted to show. Of course, much more work must be done before such a conclusion can be accepted. However, the writer is not able, at present, to say whether the larger area of tracheae is compensated by such factors as shown here in all woody plants. Nevertheless, it is of interest to the writer to note such divergencies as have been shown by these tracheae of apple and prune wood.

In the next paper data will be presented which will show the energy in terms of mechanics in relation to the upward flow of water. The significance of these experiments, with some of Dixon's work, will be discussed.

#### SUMMARY.

1. An attempt to determine some of the factors responsible for the lower efficiency per unit length and area, of the lumina of prune tracheae has been made.

2. The sap of apple and prune tracheae has been extracted by the 'gas displacement' method.

3. Density, viscosity, sugars, proteins, and ash content of these saps have been determined. In general, these determinations seem to indicate that the sap extracted from the tracheae of prune has greater density and viscosity and more sugars, proteins, and ash than the sap of apple

tracheae. There does not seem to be any direct correlation between the physical and chemical properties in these saps.

4. An apparatus has been constructed which permits the quantitative determinations of conductivity and resistance in the tracheae or tracheides of woody plants.

5. By means of the apparatus it was found that, comparing with standard walls of capillary tubes, that the walls of prune tracheae offered 10.75 per cent. more resistance to the flow of either of these saps than the walls of apple tracheae.

6. It was also found that prune sap offered 15.65 per cent. more resistance due to the nature of the sap itself than the apple sap.

7. In the previous paper it was shown that a unit area of prune tracheae was 49 per cent. more efficient in its conductivity of water stream than a similar unit of apple tracheae; while in this paper 26.4 per cent. more efficiency per unit length and diameter has been shown in favour of apple (due to resistance offered by walls and the flow of the sap).

8. There still seems to be 22.6 per cent. more efficiency in favour of prune than apple.

9. Tendency of regulating conduction stream, has been pointed out in these two kinds of tracheae. It seems that larger tracheae may be modified by other factors as viscosity of the sap and the nature of the wall.

Thanks are due to Professor D. R. Hoagland, Division of Plant Nutrition, University of California, Berkeley, for his suggestions concerning the physical and chemical analysis of saps. The writer takes great pleasure in acknowledging the kind help of Drs. J. B. Hoag and B. Dasannacharya, of the Physics Laboratory, University of Chicago, Chicago, whom the writer consulted freely. The writer records his deep appreciation of Miss Katherine Washburn, of the North-Western University, Evanston, Ill., for the drawing of the apparatus, and Miss Mary L. Lewis for reading over the manuscript.

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# Investigations on the Wilt Disease of Egyptian Cotton caused by Various Species of *Fusarium*.

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With Plates III and IV and five Figures in the Text.

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## I. INTRODUCTION.

COTTON cultivation in Egypt is the firm foundation of its prosperity. The best varieties of cotton grown in Egypt are the long staple ones, among which 'Sakel' is the principal variety. Unfortunately these varieties are seriously attacked by Wilt Disease, which is the most important disease of the cotton crop in the country. The reduction in yield of cotton caused by this disease is estimated at more than 10 per cent. of the crop.

An investigation of the disease was commenced by the writer in 1927. The experiments have been conducted under controlled environmental conditions in laboratories and glass-houses at London and Cambridge.

## II. PREVIOUS INVESTIGATIONS ON COTTON WILT DISEASE.

Several workers have investigated the wilt-producing fungi during the past forty years. Atkinson (2) attributed the wilting of cotton plants to the blocking of the vessels by the hyphae of a fungus, which he named *Fusarium vasinfectum*, Atkinson.

Smith (36) obtained the ascus stage of the fungus which he considered to be the cause of Cotton Wilt and which he named *Neocosmospora vasinfectum*, (Atk.) Smith. He failed, however, to obtain definitely successful infection with this fungus.

Orton (33) accepted Smith's identification. He induced wilting in cotton plants by inoculating the soil with pure cultures of the fungus. Later he (34) discussed the influence of climatic and soil conditions, fertilizers, &c., upon the incidence of Wilt.

Lewis (29), Gilbert (23), Neal (32), and others proceeded with the investigation of the Cotton Wilt problem in America; while Ajrekar and Bal (1), Dastur (15), and Butler (10) studied the same disease in India. Butler (11) showed clearly that the Wilt Disease is caused by a *Fusarium*,

which is capable of pathogenic action. He did not accept Dastur's suggestion regarding the accumulation of aluminium and iron salts in diseased plants as the chief cause of wilting.

Fahmy (20) studied the parasitism of single spore cultures of *F. vasinfectum* on a number of American, Indian, and Egyptian cotton varieties. Some of the Egyptian varieties were found to be very susceptible to this disease while others were highly resistant.

### III. HISTORY OF THE DISEASE IN EGYPT.

In 1902 Mosseri (31) published an account of the disease and its occurrence in Egypt. His identification of the fungus agreed with that of the early American workers, who called it *N. vasinfectum*, (Atk.) Smith.

Fletcher (22) stated that the disease known in America as the Wilt Disease was very widely distributed in Egypt, but it had not been observed before owing to its rarely causing wilting and death of the host.

In 1920, Briton-Jones (7) isolated a *Fusarium* from typically diseased plants which were obtained from two different localities in Behera Province. He demonstrated its parasitism on 'Sakel' cotton.

Fahmy (20) made a further study of the problem especially from the economic point of view. He selected wilt-resistant strains of cotton which are of the greatest interest.

### IV. DISTRIBUTION OF THE DISEASE IN EGYPT.

The Wilt Disease is at present very widely spread throughout the rich soils of Lower Egypt. In some cases the disease occurs over a large area of cotton plantations, while in others it is observed only in patches. The disease is most prevalent in the lands that belong to poor fellaheen who cultivate small acreages. It is much less destructive in larger plantations.

Infected plants, collected from every province in Lower Egypt, have been sent to the writer in England. Fungi were isolated from these infected plants and have been proved to be parasitic on the variety 'Sakel-sakha 3'.

The disease has been reported during the last few years from several localities in each province with varying severity. The intensity of attack depends upon the type and fertility of the soil and upon other environmental conditions.

Fahmy (20) states that no typically diseased plants have been found in Upper Egypt and that the discoloration within the central cylinder is peculiarly localized and of a much deeper colour than that usually found in the infected susceptible varieties in Lower Egypt.

Infected plants have been sent to the writer from different localities in Upper Egypt and the roots of some of these on examination were

found to show the characteristic discoloration of typically diseased plants. Fungi were isolated from plants collected from three provinces. In two cases out of three the organisms proved to be highly parasitic, exactly in the same manner as in Lower Egypt. The fungi obtained were, however, isolated from varieties labelled 'Sakel', 'Ashmouni', and 'Zagora', the last two of which are supposed to be resistant.

Consequently the writer believes that parasitic forms of the fungi are present in particular localities in Upper Egypt and that both susceptible and resistant varieties may be invaded by the fungi under favourable conditions.

## V. SYMPTOMS OF THE DISEASE.

1. *In the root system.* Characteristically diseased plants show the following symptoms: the fibro-vascular bundles of the root of an infected seedling are discoloured light brown, dark brown, or sometimes blackish. This discoloration may be found both in the tap root and in the lower portions of the stem. It is always present in the form of a continuous line, and has never been noticed in separate streaks as is the case in mature plants.

The tap root is generally reduced in length and thickness and the lateral roots are less abundant than in healthy plants, especially if the seedlings are grown in soil at a high temperature (25°–30° C.). The root-tip is sometimes light brown in colour. The development of the root system, as a whole, is badly affected by this disease and, under conditions favourable to the causal organism, a rotting of the roots may take place (Pl. III, Fig. 1).

2. *In the stem.* The vascular tissues of the stem of a diseased seedling show a brown discoloration, which is very similar to that described in the infected root. This discoloration extends from the tap root upwards, and the height to which it extends depends upon the age of the seedling and the severity of attack. As a rule, after the falling off of the cotyledons or leaves of a diseased plant, the stem withers, dries up, and finally dies. At this stage it becomes brittle and blackish in colour.

3. *In the foliage.* A characteristic irregular yellowing of the cotyledons and leaves is a good indication of Wilt Disease. This yellowish appearance, which is called 'mosaic', starts at the corner of one side of the leaf and it then spreads until it covers the whole surface (Pl. III, Fig. 2). A few days later, the leaf margin begins to shrivel, turns brown, dries up, though not invariably, and finally falls off.

The appearance of yellowing of the leaves is partly dependent on various factors, mainly moisture, temperature, and reaction of the soil. The type of 'mosaic' obtained under laboratory and glass-house conditions in England is sometimes the same as that obtaining in the field in Egypt. In other cases, however, especially when plants are grown in English soil,

the yellowing may be confined to the midribs of the cotyledons and appears in the form of lines instead of a network.

A general light yellow discoloration involving the whole leaf-surface has also been observed (Pl. III, Fig. 3). The 'mosaic' is only observed under certain favourable environmental conditions. For this reason its absence does not necessarily indicate the healthy state of the plant. The sure test of fungal attack is the discoloration of the root.

## VI. ISOLATION OF FUNGI FROM INFECTED COTTON PLANTS GROWN IN VARIOUS PROVINCES AND THE STUDY OF THEIR PATHOGENICITY.

During the earlier part of this investigation in London (1927), the writer isolated a *Fusarium* from the typically discoloured root of a cotton plant. This *Fusarium*, which will be referred to as 'C', was found to be entirely different from the *Fusarium* cultures which were sent to the writer through the kindness of Dr. T. Fahmy and which were called 'A' and 'B' (Mallawi). Great differences were noticeable both in morphological features and physiological behaviour of these forms, and these suggested to the writer the possibility of there being various *Fusaria* capable of attacking the Egyptian cotton plant.

Consequently fungi have been isolated from various localities of five provinces in Lower Egypt, namely, Gharbia, Behera, Sharkia, Dakahlia, and Caliubia, and from three provinces in Upper Egypt, namely, Giza, Beni-suef, and Quena.<sup>1</sup>

Small portions of the roots, about half an inch long, were cut off and carefully washed. The bark was peeled off these portions, washed again in several changes of distilled water, and the surface sterilized with mercuric chloride (1:1000). They were rinsed again in sterile distilled water and dipped in 95 per cent. alcohol, flamed, and quickly dropped into a sterilized tube containing moist cotton wool.

After twelve days' incubation at 25°C., the mycelial growth in each tube was examined microscopically and pure cultures of *Fusarium* were obtained. Subcultures on potato extract agar were made from one tube of each series.

It is interesting to note that all the plants used for isolation from Lower Egypt were 'Sakel', which is known to be very susceptible to this disease, while the plants collected from three localities of Upper Egypt were different varieties. Those from Matania were labelled 'Zagora', those from Beni-suef were 'Zagora', and the plants of Isna were 'Ashmouni'. These other varieties have hitherto been believed to be highly resistant.

All the *Fusaria* isolated by the writer and cultures of 'A' and 'B' (Mallawi) were tested for pathogenicity. Two cultures of each batch were

<sup>1</sup> The specimens were collected from the various provinces by the agricultural staff.

used for inoculating the soil of each pot (5–6 in. in diameter). The two cultures were broken up in 50 c.c. of distilled water and made into a suspension, and this was thoroughly mixed with the soil.

English loam soil was used. It was considered unnecessary to sterilize it. In the first place it was free from these particular organisms, as was proved by the controls remaining healthy. Secondly, if the soil is sterilized, its surface becomes covered with various moulds and other fungi which cause rotting and death of many seedlings under humid conditions.

Infected and control pots were sown with 'Sakel-Sakha 3' cotton, twenty-five seeds being as a rule set in each pot. Before sowing, the seeds were delinted and sterilized by soaking them in concentrated sulphuric acid for about ten minutes, and were then washed in several changes of distilled water. It was found that this treatment with sulphuric acid helped considerably the germination of the seeds. The pots were placed in a greenhouse heated to about 24° C.

Precautions were taken to avoid external infection by insects. The seedlings in each pot, after two weeks' growth, were pulled up and the roots were examined macroscopically for the presence of the organisms by cutting longitudinally. Discoloration seen in the central cylinder indicated infection. Re-isolation was made from every root that showed discoloration and in every case a pure culture of *Fusarium* was obtained. The 'mosaic' symptoms appeared on numerous plants which were undoubtedly infected.

A second attempt was made to isolate the fungus from another infected plant from Beni-suef which was typically discoloured. The only fungus isolated was a *Fusarium*, which again proved to be parasitic on 'Sakel'. This will be referred to in this paper as 'D' 1. The title 'D' will be given to the first organism isolated from Beni-suef, 'E' to that from Matania, and F to that from Isna.

In 1928, the writer received from Egypt fresh cultures of 'A' and 'B' (Mallawi) and two new cultures, which were originally isolated from Upper Egypt. They will be referred to as 'B' (Assiut) and 'B' (Beni-suef). Subcultures from these in addition to all the Upper Egypt *Fusaria* isolated by the writer during 1929 were used to carry out a series of infection experiments.

The experiment was repeated twice in exactly the same manner. Twenty-five seeds of 'Sakel-sakha 3' were sown per pot in the first instance and 30 seeds in the second. After two weeks' growth in the glass-house at about 24° C., all seedlings were pulled up and examined. Table I gives the combined results of the first and second sowings.

From that Table it is clearly seen that:—

(1) 'A' and 'C' isolated from Lower Egypt, and 'B' (Assiut), 'D', 'D' 1, and 'E' from Upper Egypt are strongly pathogenic.

(2) 'F', 'B' (Mallawi), and 'B' (Beni-suef), all from Upper Egypt, are non-parasitic.

(3) The percentages of diseased plants indicate that the infective power of the fungi varies.

TABLE I.

*Parasitism of the Various Fusaria.*

Fungus.	Locality.	No. of Plants produced.	No. of Plants healthy.	No. of Plants diseased.	Per cent. Diseased.
'A' <sup>1</sup>	?	54	10	44	81.4
'B' (Mallawi)	Mallawi	49	49	0	0
'B' (Assiut)	Assiut	44	17	27	61.3
'B' (Beni-suef)	Beni-suef	47	47	0	0
'C' <sup>2</sup>	?	53	10	43	81.1
'D'	Beni-suef	42	12	30	71.4
'D' <sub>1</sub>	" "	54	16	38	70.3
'E'	Matania	52	13	39	75
'F'	Isna	42	42	0	0
Control	—	51	51	0	0

Fahmy (20) mentions the lack of pathogenic power of the fungi isolated from several localities in Upper Egypt, but the results indicated in the previous table show clearly that the parasitic forms are present in certain localities of Upper Egypt and that under certain favourable conditions invasion of the host may take place. Moreover, in spite of the characteristic type of discoloration observed in all the roots employed for isolation, the plants were labelled 'Sakel' in the case of Beni-suef and 'Zagora' in the case of Matania. While the former variety is susceptible, the latter is supposed to be resistant.

During this investigation it was noticed that whenever the pathogenic fungi were grown on 1-2 per cent. malt-extract agar at about 25° C. the colour of the mycelium was invariably pinkish, while that of the non-pathogenic forms, under similar conditions, was always cream white.

It has also been observed that the shape and size of microconidia of the non-parasitic *Fusaria* differ from those of the parasitic ones. In the former they are larger in size and with rounded ends, while in the latter they are smaller and with more pointed ends. These characters hold good also for the parasitic and non-parasitic *Fusaria* of Upper Egypt.

*The Parasitism of the Fungus isolated from Cotyledons showing 'Mosaic'.*

The cotyledons of three different plants showing the typical 'mosaic' appearance were cut into small portions. These portions were washed and the surface sterilized with mercuric chloride (1:1000) as previously

<sup>1</sup> 'A' was originally isolated from Lower Egypt, but the locality is unknown to the writer.

<sup>2</sup> 'C' was isolated by the writer in 1927 from an unknown locality in Lower Egypt.

described. They were transferred to plates and tubes containing various media and incubated at 25° C. for three weeks. In one case only out of ten, white growth appeared at the end of the first week and was found to be *Fusarium*. Subcultures were made on acid potato plugs and infection experiments were carried out in an incubator and a glass-house at about 27° C. After two weeks' growth, the seedlings were removed and examined. Of the 20 'Sakel' plants which grew in the incubator, 4 were healthy, 6 were discoloured, and 10 were dead. In the glass-house 28 plants grew, 10 of them being diseased. Re-isolations were made from the discoloured roots and pure cultures of *Fusarium* were obtained.

It is thus concluded that the *Fusarium* isolated from the cotyledons showing the 'mosaic' appearance is parasitic on 'Sakel'. This confirms Fahmy's result (20).

*Separation of Saltants thrown by 'A' and 'B' (Mallawi) and the Study of their Parasitism*

During the course of cultural work, 'A', which had been cultivated from a single spore, and 'B' (Mallawi) showed evidence of mixture each with a saltant. The appearance of cultures showing sectoring is illustrated in Pl. III, Figs. 4 and 5. Pure cultures of the saltants were obtained by the hyphal tip method of Brown (4). The saltant strains will be referred to as 'A' 1 and 'B' 1 respectively.

An infection experiment was carried out in incubators to test the parasitism of 'A' 1 and 'B' 1 as well as that of the two parents. The saltant 'A' 1 was found to behave in a manner identical with that of the parent. In fact the results indicated that the saltant gave a slightly higher percentage of infection. As to 'B' (Mallawi), neither the saltant nor the parent caused the infection of 'Sakel' or 'Ashmouni' cotton plants. The experiment was repeated and similar results were obtained.

## VII. THE FUNGI THAT CAUSE COTTON WILT AND THEIR IDENTIFICATION.

It has not been the object of the writer to make an extensive study of the taxonomy and morphology of the fungi that cause the Wilt Disease of Egyptian cotton. A brief description, however, of the causal organisms will be given below.

According to Wollenweber and others (42), they are included in the section 'Elegans' of the genus *Fusarium*.

*Description of spores.* Three different kinds of spores, namely, microconidia, macroconidia, and chlamydospores are frequently produced under certain favourable conditions on a variety of media. The formation of each type depends upon various factors, but mainly on (1) the chemical



composition and concentration of the medium, (2) temperature, (3) moisture, (4) age of culture, and (5) the reaction of the medium.

Generally the 'micro' type is the most abundant on all media at suitable temperatures, while the 'macro' and 'chlamydo' types are more dependent on the other factors mentioned above.

(a) *Microconidia*. Majority 0-1 septate, ellipsoidal, or pear-shaped, very few comma-shaped.

(b) *Macroconidia*. Generally 3 septate, sickle-shaped, with typical attenuated apex, pedicellate.

(c) *Chlamydospores*. Mostly non-septate, terminal, conidial, and intercalary, in chains or separate.

#### *Sclerotia.*

Sclerotia were noticed only once in a one year old culture tube of 'B' (Mallawi) growing on an acid potato plug.

#### *Sporodochia.*

Sporodochia are formed in cultures of both the parasitic and non-parasitic Fusaria.

#### *Mycelium.*

The hyphae are slightly branched, and show granular contents in some cases. The mycelium is very dense, cottony in nature, and compact on rich media such as Richards's solution agar and potato extract agar; thin and loose on poor or synthetic media such as 1.5 per cent. malt extract agar, cotton juice agar, beef extract agar, or Brown's medium. It is zonate (Pl. III, Fig. 7), leathery, or immersed in the substratum on certain media, as on Richards's solution agar. Saltation occurs in cultures of parasitic and non-parasitic forms.

#### *Colour Characters.*

(1) *Spores*. Spores appear colourless under the microscope, but when seen in mass (obtained by centrifugalizing) they are light greyish. They become pinkish if exposed to light.

(2) *Aerial mycelium*. Changes in colour occur, which are due to the reaction of the substratum. The colour of the mycelium is rose on acid rice, pinkish on malt extract agar (for the parasitic forms), mauve on prune agar, white on Richards's solution agar, and greyish on Brown's agar and beef extract agar.

#### *Odour.*

The odour is aromatic on acid rice. Ammonia is liberated if the fungi are grown on Richards's solution agar.

*Size of Spores.*

In order to get an accurate measurement of the spores, 100 microconidia of the cultures 'A', 'B' (Mallawi), 'C', 'D', 'E', and 'F' grown on potato extract agar for two weeks at 25° C. were measured, and the average was considered to represent the size of the spores (Table II).

TABLE II.

*Size of Microconidia of the Various Fusaria.*

Fungus.	Range of Size.	Average Size.
'A' . . .	8.7–21 × 2.8–3.5 $\mu$ .	15.7 × 3.4 $\mu$ .
'B' (Mallawi)	14.0–28 × 3.5–5.2 $\mu$ .	20.6 × 3.8 $\mu$ .
'C' . . .	10.5–26.5 × 2.8–5.2 $\mu$ .	15.8 × 3.6 $\mu$ .
'D' . . .	10.5–23.5 × 2.8–4.4 $\mu$ .	15.8 × 3.3 $\mu$ .
'E' . . .	10.5–23.5 × 2.8–4.4 $\mu$ .	16.7 × 3.5 $\mu$ .
'F' . . .	14.0–28 × 3.5–5.2 $\mu$ .	19.9 × 4.4 $\mu$ .

On examination of the above measurements one may conclude that: (1) The average size of microconidia of 'A', 'C', 'D', and 'E' is practically identical. (2) The average size of 'B' (Mallawi) and 'F' is also similar. (3) The average spore-size of 'B' (Mallawi) and of 'F' is greater than that of 'A', 'C', 'D', or 'E'; that is, the spores of the non-parasitic types under the above-mentioned conditions are larger than those of the parasitic strains.

*Identification of the Causal Organisms.*

Fahmy (20) showed that the causal organism differs both in cultural characters and parasitism from the fungi causing Cotton Wilt in America and India. He accordingly suggested the name of *Fusarium vasinfectum Egyptiacum*.

Bewley (8) isolated four species of *Fusarium* from wilted tomato plants. By performing inoculation experiments under various conditions of temperature, humidity, and light, he found that two species never produced wilt, and these he regarded as saprophytes. The other two were strongly parasitic.

Similarly, Dowson (16) proved that the Wilt Disease of carnations is due to at least two species of *Fusarium*. A third species of *Fusarium* was weakly parasitic to shoots.

During this investigation, the writer noticed considerable differences in morphological, physiological, and biological features between the various *Fusaria* from Lower and Upper Egypt. Cultures were, therefore, sent to Professor Wollenweber, which he kindly examined and identified as follows:

(1) 'A', 'B' (Assiut), and 'D' are *F. orthoceras*, App. et Wr.

(2) 'C' is *F. vasinfectum*, (Atk.) var. *inodoratum*, Wr.

(3) 'E' is *F. angustum*, Sherbakoff.

(4) 'F' is *F. solani*, (Mart. pr. p.) App. et Wr. (non-pathogenic).

The organisms causing Cotton Wilt in Egypt will, therefore, be referred to in this paper as *Fusarium* spp.

### VIII. THE CULTURAL CHARACTERS OF THE FUNGI.

The cultural characters of these fungi were studied on a number of media varying greatly in nutritional value from the poorest, 1.5 per cent. non-nutrient agar to the richest, 2 per cent. glucose potato agar.

The media used for this study were as follows :

(1) Non-nutrient agar, (2) Malt extract agar, (3) Beef extract agar, (4) Cotton plant extract agar, (5) Dox's agar, (6) Brown's synthetic agar, (7) Rice meal agar, (8) Oatmeal agar, (9) Richards's solution agar, (10) Glucose potato agar.

The various media were prepared in the usual way, and were solidified by the addition of 1.5-2 per cent. agar. The cotton medium was prepared by extracting the juice under pressure from steamed seedlings three weeks old. 600 c.c. of this juice was made up to a litre by the addition of distilled water.

The inoculated plates were incubated at 25° C. for three weeks, and observations on the growth of the mycelium, its colour, the occurrence of saltants, and spore-formation were made each week. The *Fusaria* used for this study were 'A', 'B' (Mallawi), 'C', 'D', 'E', and 'F'. The cultural characters on the various media are briefly summarized as follows :

#### I. *Morphological Characters.*

(a) *Growth of mycelium.* The mycelial mat formed by the growth of fungi is thin and smooth on poor media, such as non-nutrient agar and 1.5 per cent. malt extract agar ; if aerial mycelium is formed, it is usually fluffy as on Brown's synthetic agar and cotton juice agar. On media rich in nutrients (particularly carbohydrates), growth is thick and the aerial mycelium is dense and compact in the form of a strong felted mass, as on Richards's solution agar and glucose potato agar. Aerial mycelium is sometimes formed in tufts or patches, while sometimes it covers the whole colony.

The formation of a layer of mycelial web, leathery in nature, occurs close to the upper surface of certain media, Richards's solution agar being a good example. On this medium the mycelial web, together with the substratum, becomes wrinkled and cracked.

Practically all the *Fusaria* studied formed what might be called 'false sectors', which appear clearly on both the upper and lower surfaces of Richards's solution agar, as illustrated in Pl. III, Fig. 8. An experiment

was made to determine whether these sectors were due to saltation. Six Petri-dishes of Richards's solution agar were inoculated from one culture as follows: Plate No (1) from the centre of the original culture; Nos. (2) and (3) from two different places on one sector; No. (4) from the line separating two sectors; and Nos. (5) and (6) from two different places on another sector. In all the plates the growth of the fungus was identical with the original culture, especially in the formation of these 'false sectors'. This was done in the case of 'A' and 'B' (Mallawi), and similar results were obtained. These 'false sectors' are probably due to the effect of fungal growth on the substratum.

(b) *Colour*. The colour of the colony and substratum varied according to the composition of the medium. The colony was pale cream, white, pinkish, mauve, grey, brownish, or greenish. A mixture of more than one of these colours were observed in one plate.

(c) *Saltation*. Saltation occurred more clearly and frequently on certain media such as malt extract, oatmeal, rice meal agar, &c., than on others. The saltation may vary from the simplest type, one sector being formed, as shown in Pl. III, Fig. 6, to the more complicated cases illustrated in Pl. III, Figs. 4 and 5.

While one or more of these *Fusaria* formed saltants on certain media, others failed to do so on the same media under similar conditions. From this it would appear that the medium which is suitable for producing saltants from one of these *Fusaria* may not necessarily be suitable for the saltation of others.

Moreover, it was often noticed that not every plate containing the same medium, and inoculated from the same culture, produced saltants. The reason for these exceptions is not known.

(d) *Spores*. The formation of one or more of the three types of spores is dependent on the composition of the medium and the age of the culture. In the majority of cases, spores were formed abundantly after two or three weeks' incubation. On most media microconidia occurred in great quantity. Macroconidia were formed less abundantly, and generally only after three weeks at 25° C. Chlamydospores were formed terminally or in an intercalary manner. In the latter case, they were formed either between two septa in a hypha or within the spores, usually the macroconidia.

(e) *Zonation*. This feature appeared occasionally on certain media, e.g. malt extract, beef extract, and glucose potato agar.

## 2. *Influence of Temperature on Growth.*

(a) *Optimum temperature for growth*. Cultures of 'A', 'B' (Mallawi)<sup>1</sup> were grown on a variety of media in incubators at the following tem-

<sup>1</sup> 'C' had not been isolated at the time these experiments were carried out.

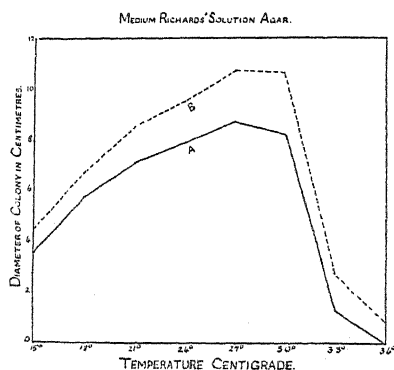
peratures: 18°, 21°, 24°, 27°, 30°, 33°, and 36° C., in addition to others at laboratory temperatures ranging round 15° C.

A typical set of results is shown in Table III, which gives the diameter of the colonies in centimetres at different temperatures as measured at two-day intervals. The medium was Richards's solution agar. The figures in the table are the averages of two readings made on each culture of a duplicate set.

TABLE III.

*Diameters (cm.) of Colonies of 'A' and 'B' (Mallawi) at Two-day Intervals.*

Fungus.	15°.	18°.	21°.	24°.	27°.	30°.	33°.	36° C.
'A'	0.35	0.75	1.9	2.2	2.4	2.1	0.60	No growth
'B'	0.65	1.3	1.7	2.0	2.45	2.5	0.80	" "
'A'	1.7	2.8	3.55	4.0	4.5	4.25	1.0	" "
'B'	1.9	3.1	3.7	4.4	5.5	5.5	1.6	" "
'A'	2.5	4.3	5.35	5.9	6.7	6.35	1.3	" "
'B'	3.3	5.0	6.4	7.2	8.5	8.4	2.0	0.7
'A'	3.5	5.75	7.0	7.85	8.0	8.2	1.3	No growth
'B'	4.5	6.75	8.5	9.5	10.75	10.65	2.7	0.85



TEXT-FIG. 1. Relation between temperature and growth after eight days' incubation.

It is clear from Text-fig. 1 that the optimum temperature for 'A' and 'B' (Mallawi) lies in the neighbourhood of 27° C., and that the latter continues to grow at a higher temperature than the former. It is also seen that 'B' (Mallawi) grows more rapidly than 'A' over the whole range of temperatures tested.

The growth-curves of both *Fusaria* on the other media were essentially similar to those illustrated in Text-fig. 1. In all cases 'B' (Mallawi) proved to have a greater growth rate and a higher temperature range than 'A'.

(b) *Maximum temperature for growth.* Cultures of 'A', 'B' (Mallawi), and 'C' were grown on Richards's solution agar in incubators at the

following temperatures: 30°, 35°, and 40° C. Duplicate Petri-dishes, inoculated with each culture, were kept at these temperatures, and the diameter of each colony was measured at intervals of two days. The mean diameter in centimetres one week after inoculation at these temperatures showed that the maximum temperature for the growth of the three fungi lies between 35° and 40° C.; at the latter temperature no growth occurred. In order to determine the maximum point more accurately, cultures were placed in incubators at 36°, 37°, 38°, and 39° C. for about two weeks, at the end of which period the plates inoculated with 'A' and 'C' did not contain any growth, while those inoculated with 'B' (Mallawi) contained growth at 36°, 37°, and 38° C., but not at 39° C. The maximum point, therefore, for 'A' and 'C' is 36° C., and for 'B' (Mallawi) is 39° C. The plates of 'A' and 'C', which had been kept at temperatures from 36°–40° C., and those of 'B' (Mallawi) which had been kept at 39° and 40° C., were then transferred to an incubator at 25° C. After one week the plates inoculated with 'A' and transferred from 39° C., and those of 'C' transferred from 40° C., did not show any growth, while the rest of both 'A' and 'C' contained growth; therefore 'A' and 'C' are killed if kept for two weeks at 39° C. and 40° C. respectively. 'B' (Mallawi) is not killed if kept for the same period at 40° C.

The higher maximum point for the growth of the non-pathogenic type 'B' (Mallawi) is of interest in connexion with its occurrence under the higher temperature conditions prevailing in Upper Egypt.

(c) *Minimum temperature for growth.* Cultures of 'A', 'B' (Mallawi), and 'C' were put in cool chambers, which were kept constant at 1°, 3°, 5°, and 10° C. for seven days. Growth was comparatively slow at 10° C., and readings were practically identical for all cultures after seven days. There was no growth at 1°, 3°, and 5° C. The minimum temperature for growth apparently lies between 5° and 10° C.

Similar cultures were put at –5°, –10°, –20°, and –25° C. for two weeks. They were then transferred to 20° C. and, after a few days, growth was seen to be normal in all cultures. These fungi, therefore, are not killed if kept at –25° C. for two weeks.

From these results it is apparent that the minimum, optimum, and maximum temperatures of the Egyptian Cotton Wilt fungi in culture are almost the same as those of the Wilt organisms of American cotton, tobacco, tomato, flax, carnations, &c., as obtained by Neal (32), Johnson (28), Clayton (12), Jones and Tisdale (27), Dowson (16), and others.

### 3. *Influence of the pH of the Medium on Growth and Spore-formation.*

Webb (42) studied the effect of the pH value of the medium on spore-germination in culture solution. He found that a range of pH values from about 3.0 to 7.0 favoured the germination of spores of the fungi tested, and

that in the case of a species of *Fusarium*, germination was quite as good in an alkaline medium as in an acid one.

Neal (32) showed that the wilt-producing fungus of American cotton grows well over a wide range of hydrogen-ion concentrations. The best growth was obtained in culture solutions adjusted to pH 3.0-5.5.

Sherwood (37) tested the growth of *F. lycopersici* in relation to reaction, and found that the fungus grew well at hydrogen-ion concentration ranging from pH 2.8-8.4; but that the spores failed to germinate at a pH of 1.8.

Johnson (28) found that *F. oxysporum*, the Tobacco Wilt Fungus, grew best on potato agar media of pH 1.7 to 7.0

An experiment was designed with a view to studying the amount of mycelial growth and the types and abundance of spores formed on Richards's solution agar adjusted to various hydrogen-ion concentrations. After autoclaving, the pH of the medium was determined by the colorimetric method described by Clark (14) and found to be 5.0. Solutions of 1 per cent. phosphoric acid and 1, 3, and 9 per cent. sodium carbonate were used for the adjustment of the medium to various hydrogen-ion concentrations. Acid or alkali was added to six different flasks, each containing 500 c.c. of medium, in the necessary amounts to give a range of pH values from 3.0 to 9.0. The medium in each flask was poured into plates, and these were inoculated with 'A', 'B' (Mallawi), and 'C' in duplicate. The plates were placed in an incubator at 25° C., and the diameters of the colonies were measured at two-day intervals. The figures in table IV are the averages in centimetres of two readings made on each of a duplicate set eight days after inoculation.

It will be noted from Table IV and Text-fig. 2 that good growth of 'A', 'B' (Mallawi), and 'C' occurred on media of pH 4.0 to 9.0, with a maximum at 7.0. Thus, these fungi are able to grow within a wide range of hydrogen-ion concentration similar to that found for other species of *Fusarium* by various workers.

The cultures described in the preceding experiments were kept in the incubator at 25° C. for three weeks. They were then examined with a view to ascertaining the different types of spores and their relative abundance. Table V shows the effect of the reaction of the medium on spore-formation.<sup>1</sup>

From Table V the following may be noted :

(1) The reaction of the medium influences the formation of certain types of spores and their abundance.

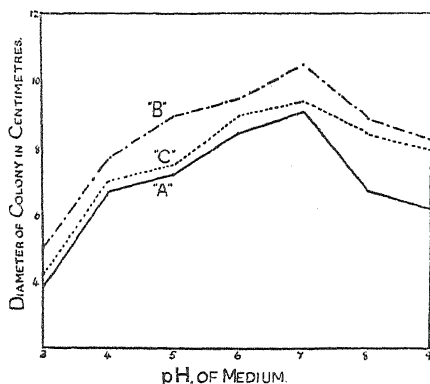
(2) Microconidia are present in abundance at all hydrogen-ion concentrations used, while macroconidia and chlamydospores are formed only on acid media (pH 3.0-6.0). The chlamydospores of 'B' (Mallawi) were also found on alkaline media.

<sup>1</sup> The extent of spore formation will be referred to in this paper as follows: × × × × = very abundant. × × × = abundant. × × = moderate. × = scarce. — = absent.

TABLE IV.

*Influence of pH of Medium on Growth.*

Fungus.	Initial pH of medium.						
	3.0	4.0	5.0	6.0	7.0	8.0	9.0
'A'	3.8	6.7	7.2	8.5	9.1	6.8	6.2
'B' (Mallawi)	5.0	7.7	9.0	9.5	10.5	9.0	8.3
'C'	4.2	7.0	7.5	9.0	9.4	8.5	8.0



TEXT-FIG. 2. Effect of pH of medium on growth.

TABLE V.

*Influence of pH of Medium on Formation of Spores.*

pH of medium.	'A'.			'B' (Mallawi).			'C'.		
	Micro.	Macro.	Chlamydo.	Micro.	Macro.	Chlamydo.	Micro.	Macro.	Chlamydo.
3.0	x x x	—	x	x x x	—	x x	x x x	x	x
4.0	x x x	x	x	x x x	—	x x	x x x	x	x
5.0	x x x	x x	x x	x x x	x	x x	x x x	x x	x x
6.0	x x x	x	—	x x x	—	x x x	x x x	x	x x
7.0	x x x	—	—	x x x	—	x x x	x x x	x	—
8.0	x x x	—	—	x x x	—	x x x	x x x	—	—
9.0	x x x	—	—	x x x	—	x x x	x x x	—	—

4. *Influence of Light on Growth and Spore-formation.*

Four plates of Richards's solution agar were inoculated with 'A', 'B' (Mallawi), and with 'C'. Two plates of each batch were wrapped in black paper, and the rest of the plates were exposed. Both series were placed in two large Petri-dishes, which were kept at laboratory temperature (15°–18° C.) near the window throughout the experiment. Measurements of diameters of colonies were made at the end of the first and second weeks. The experiment was repeated in exactly the same way, and the readings of both experiments showed that there was a tendency for better growth in the dark than in the light.



The colour exhibited in all cultures containing thick aerial mycelium, which were exposed to the light, was pale pinkish after a certain length of time. This pinkish colour is probably due to the colouring in the spore masses, which are produced in light. All cultures left in the dark and forming profuse aerial mycelium were white.

Thirty-three days after inoculation these cultures were examined to determine the types of spores formed and their abundance. It was found that spores were formed in the light much more abundantly than in the dark. At laboratory temperature, the type generally formed was the micro spore; practically no macroconidia were formed in any culture, while chlamydospores were present in moderate quantity in 'B' (Mallawi) only.

#### IX. THE INCUBATION PERIOD OF *F. ORTHOCERAS*, APP. ET WR., IN THE HOST.

Fahmy (20), considering the 'mosaic' symptom as the culmination of the incubation period, found that it took the plants from ten to fifty-three days to show any mottling. This variability in the incubation period was due to the time of year at which the experiments were carried out.

Elliot (19) stated that the seedlings of American cotton varieties growing in infected soil began to show the effects of inoculation eight days after planting.

Tisdale (38) found that the incubation period of Cabbage Yellows at 26°-32° C. was seven days.

Walker and Tims (40) showed that the first sign of wilting of onions caused by *F. cepae* was observable on the tenth day at 26° and 30° C.

The incubation period, as referred to by the writer, was reckoned from the time of germination of the seed until the appearance of discoloration in the vascular bundles of the roots, and not that of the development of 'mosaic', which appears at least four or five days after the discoloration becomes apparent in the vessels.

An experiment was carried out to ascertain the incubation period of *F. orthoceras*, App. et Wr., at different soil temperatures. It was conducted in hot-water tanks, which kept the soil temperature fairly constant at 20°, 25°, and 30° C. respectively. The average temperature in the laboratory during the experiment was about 18° C. Thirty seeds of 'Sakel' were sown in each infected pot, and three of these pots were placed in each tank. After the plants emerged from the soil, ten seedlings were pulled up from the pots in each tank every day. The roots were examined, and discoloration of the vessels indicated infection.

The first sign of discoloration was observed eight days after sowing, at 20°, 25°, and 30° C. At all three temperatures nine plants out of ten

were discoloured. Re-isolation was made from every discoloured root, and a pure culture of *Fusarium* was obtained in each case. Thus if two to three days are allowed for seed-germination, the incubation period of this fungus at these temperatures is from five to six days. Repetition of the experiment confirmed this result.

A correlation was noticed between air temperature and the progress of the disease. At low air temperatures the invasion of the fungus is greatly retarded, while at high air temperatures the disease progresses very rapidly, provided the soil temperature is favourable for fungal attack. Tims (39) obtained similar results with regard to the development of Yellows in cabbage seedlings, caused by *F. conglutinans*.

#### X. INFLUENCE OF CERTAIN ENVIRONMENTAL FACTORS ON THE INFECTIVE CAPACITY OF *F. ORTHOCERAS* AND ON THE GROWTH OF COTTON SEEDLINGS.

##### 1. *Temperature.*

There is an obvious correlation between the environmental conditions and the occurrence and severity of *Fusarium* diseases. In many of these, the temperature relationship is frequently the determining factor in their development. It is a recognized fact that the majority of wilts, due to *Fusarium* spp., develop in most destructive form during periods of hot dry weather and at a high soil temperature.

Haskel (25) found that hot weather caused extensive development of Potato Wilt, due to *F. oxysporum*. Wilt was reduced by shade, which has a large controlling influence upon soil temperature.

Jones and Tisdale (27) found that the lower critical soil temperature for the development of Flax Wilt caused by *F. lini* was about 14° C., and the optimum about 24°–28° C.

Gilman (24) showed that a close correlation existed between high soil temperature and the incidence of Cabbage Yellows, due to *F. conglutinans*. He found that the plants grown in infected soil in a warm glass-house contracted Yellows, whereas those in a cooler house remained healthy. On transferring pots from the cooler to the warmer house, the development of disease was stimulated. Under field conditions the disease occurred most seriously during hot dry summers.

Tisdale (38) states that, during hot seasons, even the resistant strains of cabbage such as 'Wisconsin Hollander' may show in a considerable percentage of cases early symptoms of the disease.

Walker and Tims (40) found that *F. cepae* is capable of producing disease in the onion at a range of 15°–32° C., but within these limits there is an optimum at about 28° C. and a gradual retardation of incidence as the temperature decreases. The disease is completely inhibited at 12° C.

Johnson (28) showed that the most favourable temperature for infection and progress of the *Fusarium* Wilt of tobacco was between  $25^{\circ}$  and  $31^{\circ}$  C.

Clayton (12) found that the *Fusarium* Wilt of tomato is most destructive at soil and air temperatures of  $25^{\circ}$ – $31^{\circ}$  C.

Neal (32) noted that, in various localities of the United States, Cotton Wilt is usually more prevalent during prolonged periods of hot dry weather.

In view of the importance of the temperature factor and of the contrast in climatic conditions in Lower and Upper Egypt, it was considered necessary to study the temperature relationships of the Egyptian Cotton Wilt Disease.

(a) *Effect of soil temperature on parasitic attack.* This was studied under laboratory conditions by growing plants in pots placed in incubators and hot-water tanks, as well as in heated glass-houses. A range of incubators at three-degree intervals from  $21^{\circ}$ – $36^{\circ}$  C. was used for this purpose. Soil was infected with the parasite, and sown with 'Sakel-sakha 3' and 'Ashmouni' in the manner already described, twenty-five seeds being planted in each pot. Uninoculated pots, serving as controls, and inoculated ones were kept at each temperature. Two weeks after sowing, all plants were pulled up and examined. The results of this experiment are tabulated below.

TABLE VI.

*Effect of Soil Temperature on Parasitic Attack.*

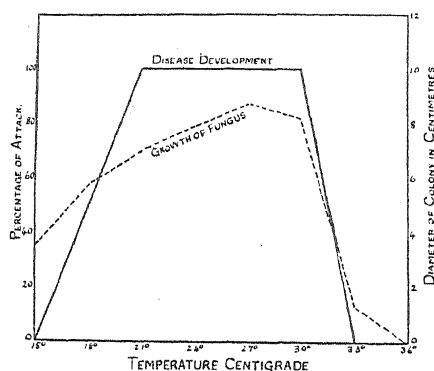
Temperature.	'Sakel'.				'Ashmouni'.			
	Infected.		Control.		Infected.		Control.	
	No. of Plants Healthy.	No. of Plants Diseased.	No. of Plants Healthy.	No. of Plants Diseased.	No. of Plants Healthy.	No. of Plants Diseased.	No. of Plants Healthy.	No. of Plants Diseased.
$21^{\circ}$ C.	0	20	22	0	21	0	22	0
$24^{\circ}$ C.	0	25	23	0	24	0	25	0
$27^{\circ}$ C.	0	25	25	0	22	0	23	0
$30^{\circ}$ C.	0	25	24	0	23	0	25	0
$33^{\circ}$ C.	22	0	22	0	24	0	25	0
$36^{\circ}$ C.	25	0	25	0	22	0	25	0

From an examination of the above table, the following conclusions may be drawn:

- (1) All the 'Sakel' plants were attacked at  $21^{\circ}$ – $30^{\circ}$  C.
- (2) No plants were infected at  $33^{\circ}$  and  $36^{\circ}$  C.
- (3) 'Ashmouni' plants and all controls kept perfectly healthy.

Re-isolations were made from every infected root and pure cultures of *Fusarium* were obtained in each case. The experiment was repeated several times with similar results.

In another experiment pots were kept at 15°C. (laboratory temperature) and 18°C. for two weeks, at the end of which no plants had come up at 15°C., this temperature being too low for the germination of cotton seeds under laboratory conditions. Some plants were infected at 18°C.; on re-isolation from the infected roots *Fusarium* cultures were obtained.



TEXT-FIG. 3. Influence of temperature on development of disease and on fungal growth.

Accordingly, the critical minimum soil temperature for fungal attack seems to be in the neighbourhood of 18°C. It is of interest to note that the corresponding critical minimum temperature of Tomato Wilt was shown by Clayton (12) to be 19°C., and that of Cabbage Yellows was found by Tims (39) to be about 18°C.

Similar experiments were carried out in hot-water tanks at 20°, 25°, and 30°C. under laboratory conditions and in heated glass-houses. The temperature of the latter ranged from 20°–30°C., being affected to a certain extent by external conditions, falling off, for instance, during the night. The results showed that an optimum attack is obtained in both cases at 25°–30°C.

It is a noteworthy fact that the optimum temperature for parasitic attack coincides with that of growth in culture media, as illustrated in Text-fig. 3.<sup>1</sup>

The temperature relationship for Cotton Wilt is thus in close agreement with that found by other workers for Wilt Diseases of tobacco, flax, tomato, and cabbage caused by species of *Fusarium*.

(b) *Effect of soil temperature on plant growth.* The influence of soil temperature on the development of cotton seedlings was studied by the following methods:

- (1) By determining wet and dry weights of plants.
- (2) By measurement of height of seedlings each day.

In the first method, plants were grown in pots containing English

<sup>1</sup> In Text-fig. 3 the curve for fungal growth is copied from graph 1.

loam soil, and placed in hot-water tanks at 20°, 25°, and 30° C. The soil of some pots was inoculated and that of others was not inoculated, to serve as controls. Fifteen seeds of 'Sakel' and 'Ashmouni' were planted in each pot. Two weeks after sowing the seedlings were pulled up, the roots washed, and the surface moisture was removed by filter paper. The seedlings were weighed, dried at 104° C. for twenty-four hours, and reweighed. The average wet and dry weights in grammes per plant (for ten seedlings) are given in the following table:

TABLE VII.

*Average Wet and Dry Weights of Seedlings at different Temperatures.*

Temperature.	'Sakel'.				'Ashmouni'.			
	Infected.		Control.		Infected.		Control.	
	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.
20° C.	0.60	0.05	0.75	0.07	0.74	0.11	0.70	0.06
25° C.	0.65	0.06	0.85	0.06	1.0	0.06	1.08	0.08
30° C.	0.75	0.05	0.94	0.06	0.80	0.06	0.84	0.06

To judge from Table VII, the optimum temperature for plant growth of both cotton varieties is 25°–30° C., considering the wet and dry weights and the ratio of wet weight to dry weight. This table shows also that plants growing in infected soil are lighter in weight than those growing in non-infected soil.

In the second method four seeds of 'Sakel' and 'Ashmouni' were planted in marked places in each pot containing non-infected soil, and placed in incubators and in hot-water tanks at 20°, 25°, and 30° C. The seedlings were measured every twenty-four hours for seven days after their emergence from the soil.

Measurements of plants growing in incubators and hot-water tanks gave similar indications as to the effect of soil temperature as those obtained by weight; namely, that the optimum temperature for the growth of cotton seedlings is 25°–30° C.

## 2. *Moisture Content of the Soil.*

Moisture, like temperature, is an important factor in the incidence of Wilt Disease as well as in the development of the host. The effect of soil moisture content on parasitic attack differs with various hosts. High soil moisture content favours the invasion of certain organisms causing wilts, while others are more pathogenic in soils with low moisture content.

Clayton (13) found that tomato plants, growing very rapidly under optimum moisture conditions for vegetative growth, were most susceptible to Wilt. There was a distinct falling off in the amount of disease in dry soils.

The observations of Johnson (28) on 'Tobacco Wilt' indicate that a high moisture content of the soil is not specially favourable to the disease.

Tisdale (38) states that the cabbage is not a high-moisture-loving plant, and that *F. conglomerans* is more destructive in soil with a low moisture content. He found that the percentage of Yellows and the mortality of plants were higher when the soil moisture was 19 per cent. than when 26 per cent., though 19 per cent. was the most favourable condition for the growth of the seedlings.

In the case of Cotton Wilt it was considered important to investigate the influence of the soil moisture factor, in view of the irrigation system carried out in Egypt in cotton cultivation, which results in the fields being watered to excess during the summer months.

(a) *Effect of soil water content on infection.* Experiments were made to find out the influence of soil water content on the infective capacity of the fungus in heated glass-houses at the Cambridge Botanic Gardens, as well as in constant temperature hot-water tanks under laboratory conditions.

The method of experimentation was as follows:

Glazed earthenware jars were filled with soil, the water content of which had been made up to four different percentages of its water-holding capacity. The type of soil used first was English loam.

The water-holding capacity of the soil was determined by two methods. It was found that the total water-holding capacity of 100 gm. of dry soil was 67.06 gm.

Each jar was sterilized with 95 per cent. alcohol, weighed, and filled with 600 gm. of soil. The soil water content was adjusted to four different percentages of its water-holding capacity. These were 40 per cent., 50 per cent., 60 per cent., and 70 per cent. in the various jars. Thirty per cent. was not used, as it proved to be unfavourable for seed-germination.

Soil was inoculated in the usual manner, and ten seeds of 'Sakel-sakha 3' were planted in each jar. Comparable jars with uninoculated soil were used to serve as controls. All jars were then placed in a heated glass-house at 21°–24° C. They were weighed every other day, and the loss of water through evaporation was made good. Only a very small amount of water, if any, had to be added, as the glass-house was kept damp all the time these experiments were in progress.

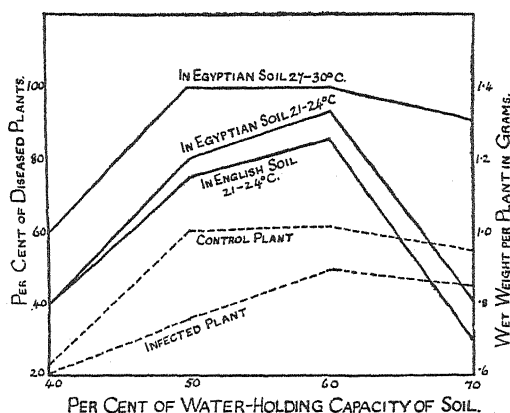
A similar experiment was conducted in another set of jars, using Egyptian clay soil. The water-holding capacity per 100 gm. dry soil was 76.7. In this case twenty seeds were planted in each jar.

After two weeks' growth, seedlings were pulled up and examined. The experiment was repeated twice in English soil and three times in Egyptian soil; the combined results are given in Table VIII.

TABLE VIII.

*Effect of Water Content of Soil on Disease Development at 21°-24° C.*

Type of Soil.	Per cent. of Moisture.	Treatment.	No. of Plants Developing.	No. of Plants Healthy.	No. of Plants Diseased.	Per cent. Diseased.
English	40	Infected	25	15	10	40
"	"	Control	22	22	0	0
"	50	Infected	16	4	12	75
"	"	Control	14	14	0	0
"	60	Infected	14	2	12	85.7
"	"	Control	19	19	0	0
"	70	Infected	17	12	5	29.4
"	"	Control	18	18	0	0
Egyptian	40	Infected	50	30	20	40
"	"	Control	52	52	0	0
"	50	Infected	51	10	41	80.3
"	"	Control	57	57	0	0
"	60	Infected	54	4	50	92.5
"	"	Control	60	60	0	0
"	70	Infected	51	30	21	41.1
"	"	Control	58	58	0	0



TEXT-FIG. 4. Effect of soil water content on disease development and plant growth.

The examination of Table VIII and Text-fig. 4 makes clear the following points:

(1) The highest degree of attack in both English and Egyptian infected soils at 21°-24° C. is at 60 per cent. of their water-holding capacity.

(2) The percentage of infected plants falls off at the upper and lower limits of the range of soil water contents tested.

(3) In the Egyptian soil used, containing 50 per cent., 60 per cent., and 70 per cent. of the water-holding capacity, attack is greater than in the English soil.

A further experiment was carried out under exactly similar conditions, except that the glass-house temperature was 27°-30° C. Egyptian soil

only was used, and twenty-five seeds were sown in each jar. This experiment was performed twice, and similar results were obtained each time. These have been combined in Table IX.

TABLE IX.

*Effect of Water Content of Egyptian Soil on Fungal Attack at 27°–30° C.*

Per cent. of Moisture.	Treatment.	No. of Plants Developing.	No. of Plants Healthy.	No. of Plants Diseased.	Per cent. Diseased.
40	Infected	43	17	26	60.4
"	Control	42	42	0	0
50	Infected	41	0	41	100
"	Control	38	38	0	0
60	Infected	45	0	45	100
"	Control	42	42	0	0
70	Infected	45	4	41	91.1
"	Control	40	40	0	0

It is interesting to note in Table IX and Text-fig. 4 that at 27°–30° C. the degree of attack is much higher than at 21°–24° C., especially in soils containing water to the extent of 40 per cent. and 70 per cent. of the water-holding capacity. All the plants were infected at 50 per cent. and 60 per cent. saturation, and attack was so severe that several seedlings were practically killed. It seems, therefore, that the raising of the temperature of the soil containing certain percentages of water has a significant effect on the incidence of disease and its destructive development. This evidence indicates that the incidence of the disease is determined both by the moisture content and the temperature of the soil.

This interesting result explains the fact that the disease is most destructive under field conditions in Egypt during the summer months, when irrigation is at its highest.

The intermediate percentages of the water-holding capacity of Egyptian soil were also tested under similar conditions and at the same time that the last experiment was being conducted. The water contents of the soils were 45 per cent., 55 per cent., and 65 per cent. of the water-holding capacity. The experiment was repeated, and the combined results are given in Table X.

To judge from the figures in Tables IX and X, the maximum degree of fungal attack, namely 100 per cent., occurs within the range of 50 per cent. to 65 per cent. of the water-holding capacity of Egyptian soil.

(b) *Effect of soil water content on the appearance of 'mosaic'.* In one of the experiments on moisture relationship, described in this chapter, it was noticed that the majority of plants growing in infected soil containing 50 per cent. and 60 per cent. of the water-holding capacity showed the 'mosaic' appearance two weeks after sowing. On the other hand, very few plants showed the same symptom in soil containing 40 per cent. of the



water-holding capacity, and none in that containing 70 per cent. Consequently careful observations were made with regard to the experiments carried out at 27°–30° C.

TABLE X.

*Effect of Intermediate Percentages of Soil Water-holding Capacity on Infection at 27°–30° C.*

Per cent. of Moisture.	Treatment.	No. of Plants Developing.	No. of Plants Healthy.	No. of Plants Diseased.	Per cent. Diseased.
45	Infected	35	8	27	77·1
„	Control	37	37	0	0
55	Infected	37	0	37	100
„	Control	40	40	0	0
65	Infected	33	0	33	100
„	Control	40	40	0	0

The following table gives the number of plants that showed the 'mosaic' symptom two weeks after sowing:

TABLE XI.

*Effect of Soil Water Content on the Appearance of 'Mosaic' at 27°–30° C.*

Per cent. of Moisture.	No. of Plants Developing.	No. of Plants without 'Mosaic'.	No. of Plants with 'Mosaic'.	Per cent. Plants with 'Mosaic'.
40	43	23	20	46·5
45	35	18	17	48·5
50	41	1	40	97·5
55	37	3	34	91·8
60	45	1	44	97·7
65	33	6	27	81·8
70	45	5	40	88·8

The figures in the above table indicate that the 'mosaic' appearance is influenced by soil moisture.

(c) *Effect of soil water content on plant growth.* The influence of soil water content on the development of cotton seedlings was ascertained by determining the wet weight per plant. The average wet weight in grammes per plant for two experiments is given in Table XII.

TABLE XII.

*Effect of Soil Water Content on Growth of Cotton Seedlings.*

40 %.		50 %.		60 %.		70 %.	
Moisture.		Moisture.		Moisture.		Moisture.	
Infected.	Control.	Infected.	Control.	Infected.	Control.	Infected.	Control.
0·60	0·63	0·75	1·0	0·88	1·06	0·85	0·95

From Table XII it is seen that the maximum wet weight for the seedlings in control and infected pots is obtained in soils possessing intermediate water contents. This agrees closely with the effect of soil water content on parasitic attack, as demonstrated in graphic form in Text-fig. 4.

The growth of the control plants at 50 per cent. and 60 per cent. of the water-holding capacity of the soil was more vigorous than that at lower and higher percentages. This conspicuous difference is illustrated in Pl. IV, Fig. 9. There was also a considerable difference in size between plants growing in the infected pot and those in the corresponding control. A good illustration is seen in Pl. IV, Fig. 10; in both pots the soil moisture was 60 per cent. of its water-holding capacity.

### 3. *Hydrogen-ion Concentration of the Soil.*

The effect of the hydrogen-ion concentration of the soil upon disease development has been taken into consideration by various investigators.

Sherwood (37) found that the highest percentage of Wilt of the tomato plant occurred in the most acid soils of the series, the pH of which varied from 5.0-8.2.

Johnson (28) found that infection of tobacco by *F. oxysporum* occurred within a wide range of soil reaction, though it was considerably greater at the higher acidities.

Egyptian clay soil was employed for the following experiments. It was alkaline in nature and its pH was 8.3. Le Geyt Worsley (30), after determining the pH of many samples of Egyptian soil, made a statement that 'all Egyptian soils, so far examined, are alkaline; that is, they have a pH above 7'. On the assumption that this is generally true, no attempt has been made to obtain naturally occurring acid soils from Egypt for this investigation, and the required types of soil reaction have been obtained by the addition of dilute hydrochloric acid and caustic soda to the Egyptian clay soil.

*Determination of soil reaction.* The pH of the soil extracts was determined by Clark's colorimetric method (14).

A sample of soil was air dried, pounded up, and passed through a fine sieve. Twenty grammes were placed in a small conical flask, and 40 c.c. of tap water were added so as to produce a suspension. Tap water was added, as it was found to have a pH of 7.0, while distilled water was acid. The flask, closed by the hand, was shaken continuously for two to three minutes, and then allowed to stand for about three minutes. The soil extract was obtained by filtration under negative pressure, and its pH was determined by the colorimetric method. In case of doubt, checks were made by using different indicators and by dilution of the soil extract; in no case were results found to vary.

(a) *Effect of soil reaction on disease development.**Experiment I.*

Egyptian clay soil of pH 8.3 was used for this experiment and the following one. To adjust the reaction of the soil to various hydrogen-ion concentrations a preliminary trial was made. A series of soil samples, weighing 200 grm. each, was used for this purpose. Ten cubic centimetres of hydrochloric acid of various strengths were added to each sample, with the object of obtaining the desired reaction: 10 c.c. of 20 per cent. caustic soda were also added to one sample to increase its alkalinity. The samples of soil were uniformly sprayed with the various solutions, by means of an atomizer, and were thoroughly mixed during the process of spraying. By such treatment the pH values of the soils were adjusted to 9.0, 8.3 (untreated sample), 8.0, 7.6, 7.3, and 7.0.

The same treatment was then extended to portions of 2,000 grm. of soil. Each portion was used to fill two pots; one was infected with the parasitic organism and the other left as a control. Thirty seeds of 'Sakel-sakha 3' were planted in each pot, and all pots were placed in a heated glass-house at 24°-27° C. for two to three weeks, at the end of which period all the plants were pulled up and examined in the usual manner. This experiment was repeated twice, and the results of the three experiments are tabulated below:

TABLE XIII.

*Effect of Soil Reaction on Disease Development.*

Initial pH of Soil.	Treatment.	No. of Plants Developing.	No. of Plants Healthy.	No. of Plants Diseased.	Per cent. Diseased.
9.0	Infected	68	39	29	42.6
"	Control	76	76	0	0
8.3	Infected	82	10	72	87.8
"	Control	86	86	0	0
8.0	Infected	84	39	45	53.5
"	Control	86	86	0	0
7.6	Infected	74	44	30	40.5
"	Control	80	80	0	0
7.3	Infected	69	60	9	13
"	Control	82	82	0	0
7.0	Infected	67	67	0	0
"	Control	60	60	0	0

The results of this experiment show that the highest degree of attack is obtained in the untreated soil of pH 8.3. It shows also the gradual reduction in the amount of disease, both with increased and with diminished alkalinity of the soil. pH 8.3 seems to be the optimum for fungal attack under the conditions of the experiment.

At the close of the experiment, the soil in each pot was thoroughly mixed and the pH was determined. As would be expected, variations

occurred on account of the washing out of acid or alkali added to the soil.

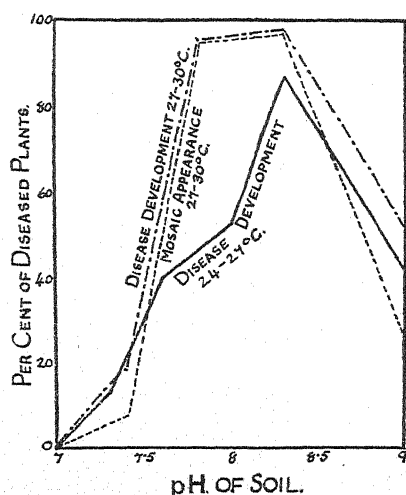
### *Experiment II.*

In preparing this experiment the samples of soil were treated in exactly the same way as in the previous one, except that pots were replaced by jam jars. The object of using jam jars was to keep the pH value of the soil constant by avoiding leaching. The reactions of the soils were adjusted to 9.0, 8.3 (untreated sample), 7.8, 7.4, and 7.0. Each jar was then filled with 600 grm. of soil of desired pH, and thirty seeds of 'Sakel-sakha 3' were planted in each jar. The jars were then placed in the glass-house at 27°–30° C. This experiment was repeated, and the results of the two experiments are given in the following Table.

TABLE XIV.

*Effect of Soil Reaction on Disease Development at 27°–30° C.*

Initial pH of Soil.	Treatment.	No. of Plants Developing.	No. of Plants Healthy.	No. of Plants Diseased.	Per cent. Diseased.
9.0	Infected	48	23	25	52
"	Control	50	50	0	0
8.3	Infected	53	1	52	98.1
"	Control	56	56	0	0
7.8	Infected	57	2	55	96.4
"	Control	56	56	0	0
7.4	Infected	51	41	10	19.6
"	Control	51	51	0	0
7.0	Infected	47	47	0	0
"	Control	55	55	0	0



TEXT-FIG. 5. Effect of soil reaction on disease development and 'mosaic' appearance.

The results indicated in Table XIV and Text-fig. 5 agree with those obtained in Experiment I. The greater severity of attack occurring is possibly due to the higher temperature (27°–30° C.) at which Experiment II was carried out.

(b) *Effect of soil reaction on the development of 'mosaic'.*

It was noticed that the reaction of the soil had a marked effect on the appearance of the 'mosaic' symptoms. The first appearance of 'mosaic' on plants growing in soil of pH 8.3 was recorded ten days after inoculation; whereas the plants growing in the soils of other pH values took a longer time to show this symptom. The numbers of seedlings exhibiting the 'mosaic' symptoms at the close of Experiment II are shown below:

TABLE XV.

*Effect of Soil Reaction on the Appearance of 'Mosaic'.*

pH of Soil.	No. of Plants Developing.	No. of Plants without 'Mosaic'.	No. of Plants with 'Mosaic'.	Per cent. of Plants with 'Mosaic'.
9.0	48	35	13	27
8.3	53	1	52	98.1
7.8	57	2	55	96.4
7.4	51	47	4	7.8
7.0	47	47	0	0

Table XV indicates that under the conditions described in the second experiment, the maximum number of plants showing 'mosaic' occurred in soils of pH 7.8–8.3.

## XI. THE TOXIC SUBSTANCE SECRETED BY THE FUNGI.

### 1. *Historical Review.*

Considerable attention has been paid by various investigators to the phenomenon of wilting of plants produced by species of *Fusarium* and other fungi.

The earlier workers such as Smith (36), Orton (33 and 34), and Dugger (18), attributed the wilting of plants, caused by various *Fusaria*, to the mechanical blocking of the vascular system by fungal hyphae. More recently the view has been held that wilting is caused by a toxic secretion from the fungi.

Brandes (6) found that the filtrate obtained from Richards's solution, in which *F. cubense*, the cause of Banana Wilt, had grown, produced wilting of buckwheat plants, bean seedlings, and banana leaves after immersion in the filtrate for various lengths of time. He attributed the wilting to a toxic substance secreted by the fungus.

Bisby (9) found that potato leaves wilted when placed in solutions in which *Rhizopus* had grown, as well as in those which had contained *F. oxysporum*, thus showing that the wilting action is not specific to the *Fusarium*. Wilting occurred also after considerable dilution, and even after boiling of the filtrate.

Fahmy (21) stated that *F. solani*, when grown under artificial conditions, produced a toxic substance which caused the wilting of cut bean stems. Wilting appeared similarly in plants placed in the boiled solutions. He found that the increase of alkalinity in the culture solution in which the fungus had grown was not responsible for the wilting.

Rosen (35) showed that the filtrates from cultures of *F. vasinfectum* growing in Richards's solution were markedly toxic to cotton plants, while the filtrates from cultures growing on a medium not containing nitrogen in inorganic form were not toxic. He stated that the change in acidity of the solutions was not a factor in rendering the filtrate toxic. He also found that the toxic properties of the filtrates of Richards's solution were not due to the increase in osmotic pressure. Rosen came to the conclusion that there were at least two toxic substances in the filtrate, one a volatile compound with an alkaline reaction, and the other an inorganic salt in the form of a nitrite.

Dowson's (16) experiments on the filtered liquid in which *F. culmorum* had grown, and in which carnation shoots were placed, showed that a toxic substance was conveyed by the transpiration current to the chlorophyll tissues, which were killed. Wilting took place in a few hours without loss of colour.

Brooks and Brenchley (3) found that silvering of the foliage of plum trees and other pathological symptoms were produced in the host on injecting the stems with a filtered extract of *Stereum purpureum*. The same results were obtained on using only the culture fluid in which the fungus had grown. When the fluids were boiled for five minutes before being injected, no silvering of the leaves occurred, but other pathological symptoms developed on the trees.

Bewley (8) found that the filtrate of fluid cultures in which *Verticillium albo-atrum* was grown contained a toxic substance, which caused wilting of tomato plants. He attempted to isolate any exo- and endo-enzymes which might be produced by the fungus and concluded that, under certain conditions, a definite exo-enzyme, which can be precipitated by absolute alcohol, was excreted and was capable of causing wilt.

Dowson (17) stated that the variety of Michaelmas Daisy 'Gladys Domellan', which was resistant to fungal attack, was susceptible to the toxin produced by *Cephalosporium* sp. in the same way as the susceptible varieties were.

2. *Preparation of Fluids used for Experiments.*

A. *Preparation of filtrates and extracts of ground mycelium.*

In practically all the experiments which follow, the fungi were grown in Richards's solution, this being the medium on which the most profuse and dense mycelial growth has been obtained in the writer's experience. A preliminary test was made to find out if sterile Richards's solution produced any wilting of cotton seedlings. The roots of seedlings were cut off under water, and the cut stems were rapidly dipped in the liquid medium. Under laboratory conditions, wilting was produced within forty-five minutes. To avoid this effect, the medium was diluted to  $4/5$  N,  $3/5$  N,  $2/5$  N, and  $1/5$  N, where N is the strength of standard Richards's solution. It was found that, when  $N/5$  solution was used, wilting entirely failed to occur within five hours, though it was pronounced after twenty-four hours. This dilute medium was accordingly chosen for growing the fungi during this part of the investigation.

The culture solution was placed in half-litre conical flasks, 350 c.c. being used in each. Nine of these flasks were sterilized and inoculated with the *Fusarium* forms 'A', 'B' (Mallawi), and 'C', using three for each culture; three uninoculated sterilized flasks served as controls. All the flasks were incubated at  $25^{\circ}$  C. for a varying length of time, according to the object of each experiment.

The cultures were then filtered under negative pressure, and the mycelial mats were collected on filter paper. For every experiment two equal portions of the filtrate from each culture were taken; one portion was boiled for about two minutes, and the other portion was left unboiled. The object of boiling was to destroy and expel all thermolabile and volatile substances. Another portion was diluted so as to make an equal volume of liquid of half the concentration.

A series of small conical flasks containing 25 c.c. of the different solutions was prepared in which to test the rate of wilting of cotton shoots.

The flasks contained the following liquids:

- |                              |                                |
|------------------------------|--------------------------------|
| (1) Filtrate unboiled.       | (4) $N/5$ Richards's solution. |
| (2) „ boiled (2 minutes).    | (5) Distilled water.           |
| (3) „ unboiled, diluted 1:1. |                                |

The mycelial mats, collected on the filter paper, were thoroughly washed with sterile distilled water. They were then pounded up vigorously with equal amounts of pure sterilized sand for about 15–20 minutes. The mixtures were extracted with distilled water and then filtered. A second filtration through a Chamberland filter was found necessary as the filtrates were turbid. The extract was used to make up a precisely similar series

of flasks to that previously described. In both cases separate series from cultures of 'A', 'B' (Mallawi), and 'C' were prepared.

*B. Preparation of precipitate obtained by addition of alcohol to filtrates and mycelial extracts.*

For the preparation of these fluids, larger amounts of filtrates and mycelial extracts were needed; and accordingly the fungi were grown on N/5 Richards's solution in litre flasks, each containing 750 c.c. of the medium. 'A', 'B' (Mallawi), and 'C' were each used for inoculating two flasks. All flasks having been incubated at 25° C. for varying periods according to the object of the experiment, filtrates and mycelial extracts were then prepared.

The fluid obtained in each case was poured into a large bottle, and small portions of 95 per cent. alcohol were gradually added, the mixture of fluid and alcohol being thoroughly shaken during the addition of the latter until the strength of alcohol was 70 per cent. At this strength all the proteins are precipitated. The solution in each bottle was allowed to stand for forty-eight hours, at the end of which all the precipitate had accumulated at the bottom of the bottle. The solutions were then filtered, and the precipitated substances were collected on the filter paper. They were scraped off with a knife and, after being air-dried, each precipitate was redissolved in 100 c.c. of distilled water.

A series of five small flasks was arranged in exactly the same way as described above for the filtrates and mycelial extracts. Six of these series were prepared, using the precipitates obtained from the filtrates and the mycelial extracts of 'A', 'B' (Mallawi), and 'C'.

*C. Preparation of an extract from young hyphae.*

An attempt was made to prepare an extract from young hyphae by the technique described by Brown (5). An abundant supply of spores was obtained by growing the fungus 'A' on Richards's solution agar, which was incubated at 25° C. for 3–4 weeks. The spores were then germinated in 50 c.c. of sterilized undiluted turnip extract and flat circular plates, 8 in. in diameter, were each sown with 5 c.c. of the spore-suspension, which was uniformly spread over the surface. After 48 hours had elapsed, the fungal material was obtained and thoroughly dried. One gramme of the dried material was ground with an equal weight of pure sterilized sand. The mixture was then suspended in 40 c.c. of distilled water for 2½ hours; being shaken occasionally during this period. The suspension was centrifugalized for 5 minutes, and the extract was divided into two equal portions. One portion was boiled for 2 minutes and the other was left unboiled. They were then tested for their capacity to cause wilting.

In all experiments which will be discussed later, 2–3 weeks old cotton



seedlings were used for the various tests, except where otherwise stated. The roots were cut off under water, and the cut shoots were rapidly transferred to the various fluids. The experiments were conducted under laboratory conditions in fairly bright light at a temperature varying from 15°–20° C.

### 3. Results obtained with the Excretory Toxic Substance :

#### A. In the Fluids in which the Fungi had grown.

##### (a) Effect of the toxin in the fluid on cotton shoots.

The filtrates used for this experiment were obtained from 3 weeks old cultures of 'A', 'B' (Mallawi), and 'C'. The varieties of cotton seedlings used were 'Sakel', 'Garofolou', and 'Ashmouni', the first two being highly susceptible, and the last resistant to disease. Two seedlings of each variety were labelled, and cut shoots were inserted in each flask of the three series. At the close of this and several other experiments of a similar nature, it was found that the effect of the filtrates of 'A', 'B' (Mallawi), (non-pathogenic), and 'C', in inducing the wilting of seedling shoots was practically the same. Further, the three cotton varieties tested were influenced in exactly the same manner in corresponding fluids. It appears, therefore, that the susceptible and resistant varieties are equally affected by the filtrates of both the pathogenic and non-pathogenic forms. A typical set of data obtained from the filtrate of 'A' is represented in Table XVIII.

TABLE XVI.

*Toxic Effect on Cotton Seedlings of the Fluid in which Fusarium 'A' had been Growing for 3 Weeks.*

Time after.	Boiled for 2 min.	Filtrate.		N/5 Rich. Sol.	Dist. water.
		Unboiled.	Diluted 1:1.		
$\frac{1}{2}$ hour	No wilting	No wilting	No wilting	No wilting	Sound
1 "	Wilting slight	Wilting slight	" "	" "	"
2 hours	Wilting positive	Wilting positive	Wilting slight	" "	"
3 "	Wilting pronounced	Wilting pronounced	Wilting positive	" "	"
5 "	Wilting very pronounced	Wilting very pronounced	Wilting pronounced	" "	"
24 "	Wilting very pronounced	Wilting very pronounced	Wilting very pronounced	Wilting very pronounced	"

From the above Table it is obvious that the filtrate has a toxic action which causes the wilting of cotton seedling shoots. When the plants were stood in the boiled and unboiled fluids, wilting was positive after 2 hours,

and pronounced after 3 hours, while all plants standing in fresh medium and in distilled water kept perfectly turgid for a much longer time. From the fact that wilting was as rapid in the boiled as in the unboiled filtrate, it would appear that this effect cannot be attributed to any volatile or thermolabile substance. After 24 hours, wilting was pronounced in every case except in that of distilled water. Wilting of seedlings placed in the filtrate of half concentration was positive after 3 hours; that is diluting the filtrate lowered its toxicity and retarded the wilting effect.

In another experiment, cut shoots of broad bean (*Vicia faba*) growing under laboratory conditions, and of dead-nettle plants (*Lamium album*), were placed with cotton seedlings in the filtrates of the fluids in which the fungi were growing. It was found that the cotton seedlings succumbed and wilted in about 2 hours, but that the broad beans and dead-nettle plants kept sound for 72 hours. It is therefore evident that, under the conditions of the experiment, the substance given off by the Egyptian Cotton Wilt fungi, which is toxic to cotton, is not toxic to certain other plants.

In another test the length of the boiling-period of the filtrates was extended to 6 minutes. The effect of these solutions was precisely the same as that of the solution boiled only for 2 minutes.

(b) *Effect of changes in the reaction of the filtrates on the production of wilting.*

In a preliminary test, it was found that the culture medium (N/5 Richards's solution), in which the fungi had grown, gave a markedly alkaline reaction after being filtered, while the medium when freshly made up was acid. Consequently, it was thought desirable to determine if alkalinity of the filtrates was directly responsible for the wilting.

Various cultures of 'A', 'B' (Mallawi), and 'C' in N/5 Richards's solution were grown at 25° C. for 1, 2, and 3 weeks. After each period had elapsed, the fluid from a culture of each type was filtered, and the pH value of each was determined. The results obtained are tabulated below.

TABLE XVII.

*The Change in the Hydrogen-ion Concentration of the Filtrates related to the Age of Culture.*

Age of Culture.	pH of Medium.	pH of Filtrates.		
	N/5 Rich. Sol.	'A'.	'B' (Mallawi)	'C'.
1 week	5.0	4.8	5.2	5.9
2 weeks	"	5.1	7.3	7.2
3 "	"	7.4	8.3	8.2

From Table XVII it is apparent that the reaction of the three filtrates was still acid after one week's incubation, but that alkalinity increased with

the age of culture. It is also to be noted that in the case of 'A' there was a slight increase in acidity of the filtrate at the end of the first week. Sherwood (37), working on *F. lycopersici*, has recorded a similar observation.

The influence of these filtrates of different pH values in inducing the wilting of cotton seedlings was tested. Cut plants were inserted in all these solutions, in N/5 Richards's solution, and in distilled water. The degree of wilting produced, and the time required to produce it, were identical in all filtrates whether acid, slightly alkaline, or markedly alkaline in reaction. It is thus clear that though the alkalinity of the filtrates increased with the age of culture, it was not directly responsible for the wilting.

As a further test, the pH values of the filtrates of 'A', 'B' (Mallawi), and 'C', three weeks old, which were 7.4, 8.3, and 8.2 respectively, were adjusted to the original pH of the culture solution, namely 5.0, by the addition of dilute hydrochloric acid, and cut stem plants were placed in the three filtrates. Wilt was produced in all cases after one hour. This is another indication that the change in reaction of the filtrate, either towards alkalinity or acidity, fails to influence the degree of wilting produced.

(c) *Effect of filtrates on plants growing in pure sterilized sand.*

As the results obtained so far had shown that a toxic substance is secreted by the fungi, which causes wilting in cut cotton shoots, it was considered worth while to study the effect of the toxin on plants growing in pure sterilized sand.

Filtrates of 'A' and 'B' (Mallawi) which had been growing in N/5 Richards's solution for 8 weeks at 25° C. were added to pots containing pure sterilized sand at the time of sowing, 50 c.c. being used per pot. The same procedure for boiling equal portions of both filtrates was adopted in this test. Care was taken to avoid the loss through drainage of the filtrates added. Pots were sown with sterilized 'Sakel' and 'Ashmouni' seed; 25 seeds being planted in each pot. Germination and growth of seedlings were fairly normal, and no wilting appeared in any pot for 2 weeks, after which period the seedlings were pulled up for examination.

From this it would appear that, under the conditions of the experiment, the amount of toxin taken up by the plants was not sufficient to bring about the wilting of the plants.

(d) *Effect of the age of the plant on the wilting response.*

In the experiments carried out so far, cotton seedlings 2-3 weeks old were employed. For this experiment, however, plants about 2-3 months old were used. The stem of each plant was about 70 c.c. high, and about

0.5–0.75 c.c. in diameter. The filtrate of N/5 Richards's solution, in which 'A' had been growing for 8 weeks at 25° C. was divided into two portions of 300 c.c. each. One portion was boiled for 2 minutes, and the second was kept unboiled. The two portions of the solution were poured into jars, and these were placed in a heated glass-house at about 27° C. along with control jars containing N/5 Richards's solution and distilled water. Plants were taken out of the pots carefully, and the adhering soil was washed away. The roots of half the number of plants were cut off under water, and two of these cut shoots, and two of the uninjured plants, were inserted in each jar containing the solutions.

It was found that no sign of wilting appeared in any case within 24 hours. After 30 hours, wilting was visible in the cut shoots which had been placed in both the boiled and the unboiled filtrates. The uninjured plants wilted after 48 hours in both solutions. After 5 days, wilting of all the plants in the filtrates was pronounced, and about this time the leaves began to fall off. The plants that had been inserted in distilled water kept turgid for about 2 weeks, after which the leaves withered and fell off. Pl. IV, Fig. 11, illustrates the toxic effect produced on plants in the filtrates, and the perfect state of those in water.

It is rather striking that the 2–3 months old plants, when stood in the filtrate of a culture that had been growing for 8 weeks, and in 12 times the volume of filtrates generally used, showed no sign of wilting before 30 hours; whereas 2–3 weeks old seedlings, placed in the filtrate of a culture that had been growing for 5 weeks, invariably wilted after 2 hours. From this it can be concluded that the age of the plant is an important factor in the action of the toxin contained in the filtrate. Hursh (26), working on a problem of a like nature, obtained similar results.

(e) *Effect of filtrates from media containing nitrogen only in organic form.*

Rosen (35) has recorded that the filtrates from solutions containing nitrogen in organic form only, such as Uschinsky's asparagin solution or peptone beef broth, in which the American Cotton Wilt fungus *F. vas-infectum* had been grown, were not toxic to American cotton varieties. It seemed worth while to determine if such was the case with the Egyptian Cotton Wilt fungi.

Cultures of 'A', 'B' (Mallawi), and 'C' were grown at 25° C. for 4 weeks on Uschinsky's and asparagin synthetic solutions, both containing nitrogen in organic form only. Filtrates were obtained from both media, and were tested in the usual manner with cut cotton seedlings for the presence of any toxic substance capable of causing wilting.

Wilt was positive after 45 minutes in the filtrate obtained from the asparagin synthetic solution, and after 2 hours in that obtained from Uschinsky's solution. It was pronounced in both cases after 2½ hours in

the boiled and unboiled filtrates. In the dilute filtrate (1 : 1), wilting was retarded.

The writer's observations, therefore, do not agree with the results of Rosen, and he concludes that, so far as Egyptian cotton varieties are concerned, cut shoots succumb to wilt in media containing nitrogen only in organic form, as well as in media containing nitrogen in the form of inorganic salts.

(f) *Determination whether the nitrite present in the filtrates of the used culture fluids is responsible for wilting.*

Rosen (35) came to the conclusion that the filtrate from Richards's solution, in which *F. vasinfectum* had grown, contained nitrite which was poisonous to cotton plants, and partly responsible for the wilting. The present writer thought that these results were open to criticism, and accordingly tests were performed with a view to ascertaining if nitrite was present in the filtrates of fluids in which the fungi had been growing for various lengths of time. Greiss's method of testing for nitrite (sulphanilic acid alpha-naphthylamine) was adopted for this purpose.

In a preliminary experiment, the nitrite test gave a positive reaction in the filtrates of cultures 'A' and 'C', and a negative reaction in that of 'B' (Mallawi), the three cultures being about three weeks old. The test was repeated, using filtrates from one week old cultures of the same fungi, and it gave a positive result in the case of 'A', and a negative one in the case of 'B' (Mallawi) and 'C'. It is obvious, therefore, that the nitrite is formed in certain culture solutions, but is absent from others. Its presence appears to depend partly upon the age of the culture.

Dilute hydrochloric acid was added gradually to the filtrates which gave the positive reaction, and the filtrates were then boiled, with the result that the nitrite present was removed. The filtrates thus treated were tested as to their toxicity to cotton plants along with the untreated filtrates which contained nitrite. Cut seedlings were inserted in the various solutions, and it was found that they wilted in every case within about the same time. The presence of nitrite in some of the solutions was not therefore responsible for the wilting.

As the pH values of the filtrates were changed by the addition of hydrochloric acid in the above experiment, they were adjusted in another test to their original reaction. It was found that the cut shoots placed in these filtrates wilted within the same time.

The toxic effect of pure sodium nitrite solutions of various concentrations was also investigated. Cut stem plants were placed in equal quantities of 1 per cent., 2 per cent., and 3 per cent. solutions of sodium nitrite, and in distilled water. The readings indicated that wilting was positive in the 1 per cent. solution within 4 hours, and very pronounced in stronger

solutions within that time. Weaker solutions were similarly tested for the same purpose. In 0.25 per cent. and 0.5 per cent. solutions, wilt was produced after 20 hours; the latter solution being almost 25 times stronger than that employed by Rosen (35), which caused wilting of the plants he used within 22 hours.

From these various experiments the writer concludes that such small quantities of nitrite as occur in the filtrates described above can have no appreciable effect in causing the wilting of Egyptian cotton plants.

*B. In the Filtrates of the Extracts obtained after Pounding up Mycelial Mats in the Liquid Medium in which they had grown.*

Cultures of 'A', 'B' (Mallawi), and 'C' growing on N/5 Richards's solution were employed for this experiment. It was found that the results obtained by inserting young cotton shoots in the filtrates of the mycelial extracts were identical with those obtained from the filtrates of the culture fluids; wilting was produced in about the same degree.

*C. In the Substances precipitated by Alcohol from the Filtrates of the Culture Fluid and the Mycelial Extracts.*

Cultures of 'A', 'B' (Mallawi), and 'C' were grown in N/5 Richards's solution, two flasks containing 750 c.c. being inoculated with each, and incubated at 25°C. for 6 weeks. The precipitate was obtained from the filtrates of the three fluids by the method described previously. The precipitate in each case was redissolved, and equal amounts of this solution were boiled, left unboiled, and diluted with water in the ratio 1:1 and 1:2. The cut stems of cotton seedlings were placed in the fluids, and observations of the time of wilting were made. It was noticed that, in the boiled and unboiled solutions, the wilt was slight after 45 minutes, positive after 1 hour, and pronounced after 1½ hours. In the diluted solutions wilting did not appear until 1½ hours had elapsed.

Similarly, a substance was precipitated from the mycelial extracts of the three above-mentioned cultures. The precipitate in each case was redissolved in distilled water and tested for toxic action. Wilting of inserted cut plants was positive in each case, but only occurred after 3 hours, a longer time than in the corresponding filtrates of the fluid in which the fungi had grown.

Diluting these solutions lowered the toxicity considerably, as wilt appeared only after 5 hours.

*D. In the Extract of Young Hyphae.*

Cut stem cotton plants, 2-3 weeks old, were placed in the extract of the young hyphae of the fungus 'A'. This extract produced wilt within an hour. On heating an equal volume of the extract, wilt did not occur

except after a much longer time. It therefore appears that the fungus 'A', when only two days old, secretes a substance which is able to cause the wilting of seedlings within about the same time as that of the filtrates obtained from fluids in which the fungus grows.

## XII. SUMMARY.

1. The Wilt Disease of Egyptian cotton is widely distributed in the Delta, the reduction in yield of cotton being estimated at more than 10 per cent. of the crop.

2. The disease causes a brown discoloration of the fibro-vascular bundles of the roots and stems of seedlings. A characteristic yellowish appearance, called 'mosaic', in the cotyledons and leaves is the first external symptom of Cotton Wilt under favourable conditions.

3. The fungi isolated from affected plants in various localities of Lower Egypt, and in three localities of Upper Egypt, proved to be highly parasitic on 'Sakel' cotton. Fungi isolated from cotton plants from three other localities of Upper Egypt were found to be non-pathogenic.

4. The fungi responsible for Cotton Wilt in Egypt are various species of *Fusarium*. Those identified so far are :

(1) *F. orthoceras*, App. et Wr. ('A' and 'D').

(2) *F. vasinfectum*, Atk. var. *inodoratum*, Wr. ('C').

(3) *F. angustum*, Sherbakoff ('E').

5. The cultural characters of these fungi have been studied extensively on a variety of media and their growth rates at different temperatures, and on media of different pH values, have been determined.

6. The incubation period in the host of *F. orthoceras*, App. et Wr., at 20°, 25°, and 30° C. was 5-6 days.

7. Fungal attack on the cotton plant was found to be severe at all temperatures between 21°-30° C. No plants were infected at 33° C. and 36° C. The minimum soil temperature for infection is in the neighbourhood of 18° C. The best growth of cotton seedlings was obtained at 25-30° C.

8. The greatest number of plants were attacked and showed the 'mosaic' appearance in soil containing water to the extent of 50-60 per cent. of its water-holding capacity. The plants were most vigorous under similar conditions of soil moisture content.

9. The highest degree of attack occurred in English and Egyptian soils of pH 7.8-8.3.

10. An historical review of investigations on the wilting of plants caused by various species of *Fusarium* and other fungi is given. Filtrates of the culture fluids in which the two pathogenic forms, *F. orthoceras*, App. et Wr., *F. vasinfectum*, Atk. var. *inodoratum*, Wr., and the non-pathogenic, *F. solani*, (Mart. pr. p.) App. et Wr., had been grown, and extracts from the mycelium of these species have been shown to cause

wilting of young cotton shoots (including the variety 'Ashmouni', which is resistant to Wilt Disease). The toxic substance in these fluids and extracts is thermostable and non-volatile. It has been shown that the presence of nitrite in the fluids is not the cause of wilting.

#### ACKNOWLEDGEMENTS.

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## EXPLANATION OF PLATES III AND IV.

Illustrating Dr. A. Fikry's paper on Investigations on the Wilt Disease of Egyptian Cotton caused by Various Species of *Fusarium*.

### PLATE III.

- Fig. 1. Right : rotting of the roots of infected seedlings.  
 Left : healthy roots of control seedlings.
- Fig. 2. The 'mosaic' symptoms on cotyledons.

Fig. 3. A yellowing covers nearly the whole surface of cotyledons.

Fig. 4. Saltation of the parasitic fungus 'A'.

Fig. 5. Saltation of the non-parasitic fungus 'B' (Mallawi).

Fig. 6. Simple type of saltation (one sector only formed).

Fig. 7. Zonation of 'B' (Mallawi).

Fig. 8. Showing 'false sectors' (upper and lower surfaces) of 'A' on Richards's solution agar.

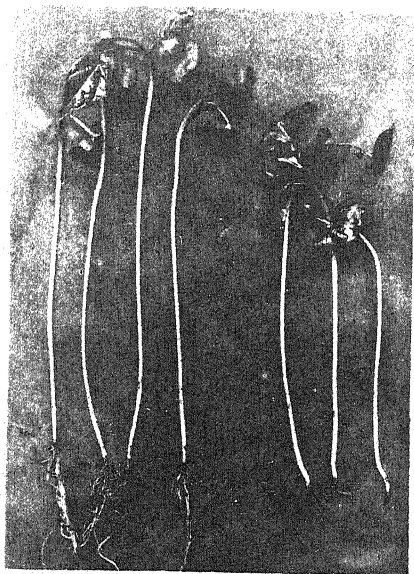
#### PLATE IV.

Fig. 9. The effect of the soil water-content on plant growth. From left to right : 70 per cent., 60 per cent., 50 per cent., and 40 per cent. of the water-holding capacity of the soil.

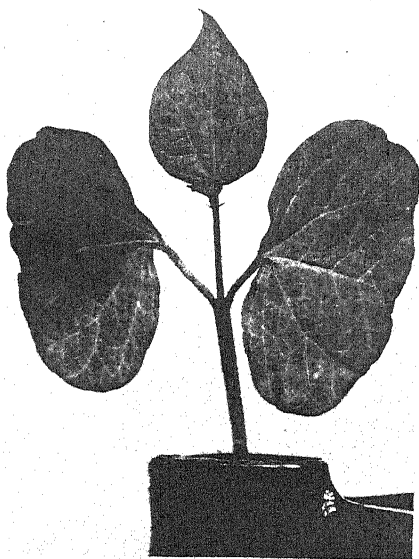
Fig. 10. The effect of infection on plants grown in soil containing water to the extent of 60 per cent. of the water-holding capacity : infected (right), healthy plants (left).

Fig. 11. The toxic effect on young cotton plants of the filtered culture medium in which *F. orthoceras*, App. et Wr., had grown. From left to right : control, unboiled filtrate, and boiled filtrate.

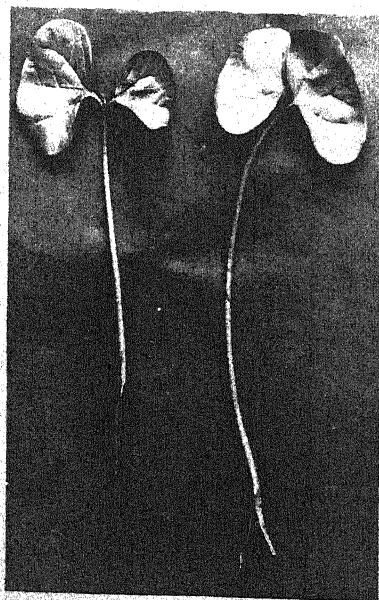




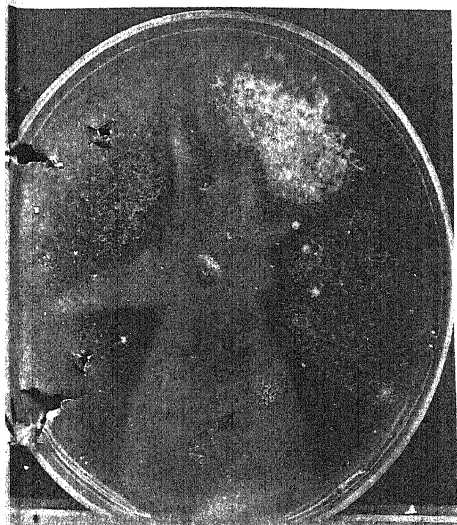
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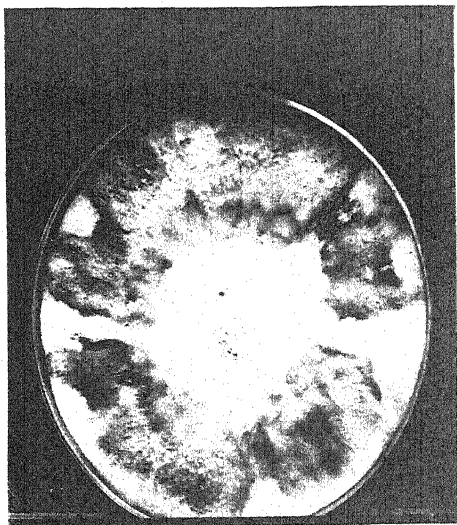
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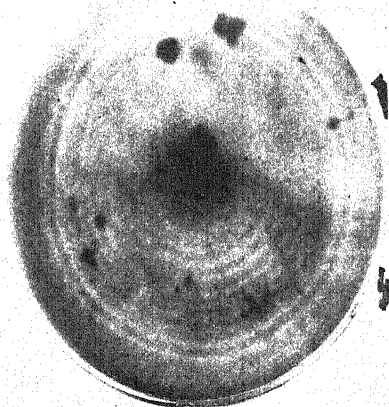
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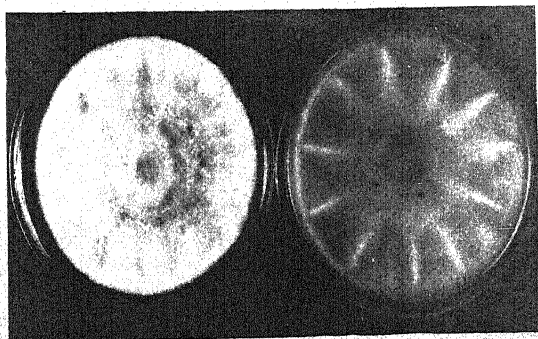
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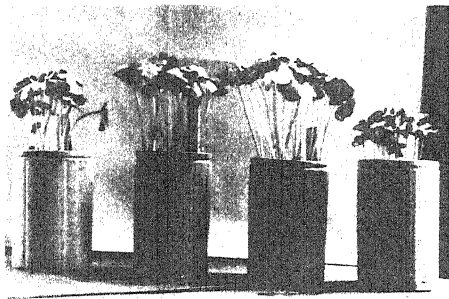
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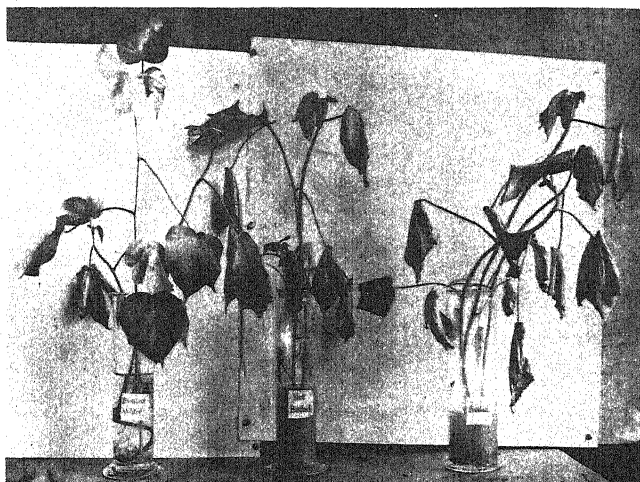




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FIKRY — COTTON WILT.





# The Fruit-body of *Polystictus xanthopus*, Fr.

BY

E. J. H. CORNER, M.A.

(*Botanic Gardens, Singapore.*)

With Plate V and seventeen Figures in the Text.

IF there exists some doubt concerning the primitive state of the fruit-body in a group of fungi, one can be sure when the hyphae which compose the fruit-body are of several different kinds that one is then dealing with a specialized organism. Admittedly, for the hyphae are most often of one kind, the criterion is of limited application, yet an exception appears in the Polyporaceae. In polypores with soft fleshy fruit-bodies, ill adapted to withstand desiccation and of short life, all the hyphae certainly are branched and septate alike, but examine the mature structure of some of the woody or coriaceous forms and it will be found that there are, as it were, distinct systems among the hyphae. Some may be branched, some unbranched; some may be thick-walled, others thin-walled; and some may be unseptate. Thick-walled unbranched hyphae may course longitudinally through the stroma and others, even thicker-walled, may branch profusely among them and weave the whole into a tough felted tissue; while in some cases thin-walled septate hyphae can also be detected with care. Such differences imply a division of labour which affects the mechanical construction and the nutrition and generation of the hyphae; such fruit-bodies in fact are the more highly specialized.

The hyphal system of the fruit-body must be considered foremost in the morphology of polypores as it will undoubtedly provide the key to a natural classification. In certain species of the genus *Fomes*, in *F. pachyphloeus*, Pat., for example, the very thick-walled, dark brown, and unbranched hyphae which occur among the smaller branching hyphae of the fruit-body are so outstanding that they are used as a diagnostic character, although their origin and destination—for they seem to end blindly in the flesh—are still to be discovered. To obtain some facts, therefore, I have examined in as much detail as possible the structure of a common species which can be grown without trouble in the laboratory; I find it unexpectedly complex.

*Polystictus xanthopus*, Fr. has been known for more than a century as a common saprophyte on sticks and branches in the tropics of the old world. There are records of the species from America in Saccardo's 'Sylloge', but Lloyd considers them erroneous (6); in his wide experience he never received specimens from thence. More recently, however, Killermann has again stated that it is pantropical (5).

For Ceylon, Petch mentions that it is an inhabitant rather of the low country (9). In the Singapore Herbarium, besides collections from many of the low-lying parts of Malaya, we have specimens from Cameron's Highlands in Pahang at 6,000 ft., and I have found it in abundance on Fraser's Hill, Pahang, at 4,000 ft. and in Negri Sembilan, on Gunong Angsi, at 2,700 ft., so that the fungus seems to be generally distributed through the peninsula regardless of altitude.

Fruit-bodies can be gathered the year round in Singapore from damp parts of the jungle, though in greatest profusion after a spell of wet weather. The primordia can easily be raised from sticks bearing the fruit-bodies if these are removed and the sticks are kept very damp under a bell-jar. I held the sticks under a running tap for a few seconds every morning or splashed them heavily with water and replaced the bell-jar for the rest of the day. A damp atmosphere does not suffice entirely for the development of the fruit-bodies, but the wood from which they grow must also be soaked with water.

In microscopical work the system of the hyphae can be followed most readily in free-hand sections of fresh material after fraying out the section with fine needles. Rather thick sections are preferable because portions of hyphae of a millimetre or more in length can then be freed under a binocular microscope. The method gives an indication also of the structure, whether fibrillar with the hyphae longitudinal, or felted from interwoven hyphae, according to the readiness with which the hyphae separate; with a very dense felted tissue no more than little chunks can be freed.

As a detailed description based on fresh material has not been published, I have drawn one from collections made in Singapore and South Johore, and on Plate V there are two photographs, taken in the jungle, to show the habit of growth of the fruit-bodies.

### *Polystictus xanthopus* Fr.

Mesopodal, coriaceous, thin.

*Pileus* 2.5—19 cm. wide, *infundibuliform* from the first, often oblique, smooth or concentrically rugulose, *glabrous, shining, very thin, tawny ochraceous with narrow darker tawny chestnut zones*, paler towards the margin, *often becoming wholly deep chestnut or bay brown, finally very dark or blackish when old*: margin white, even, entire or lobed in large specimens.

Flesh 320–600  $\mu$  thick, dry, especially tough at the base of the stem, white.

*Stem* 0.3–5.0 cm. long  $\times$  1.2–4.0 mm. thick, mostly 8–20  $\times$  2–2.5 mm., central or eccentric, *never truly lateral*, cylindric or slightly expanding upward, dilating into the pileus, apex sharply delimited, *glabrous, yellowish cream to pale ochraceous*, luteous, *often spotted ferruginous, arising from a concolorous discoid base*, 1.5–12.0 mm. wide, *velutinate at the periphery from a dense annular pile of hairs* which cracks on drying.

*Tubes* 120–140  $\mu$  long  $\times$  80–100  $\mu$  wide, with dissepiments 15–30  $\mu$  thick, *very short*, abruptly delimited from the stem, *white: mouths minute*, 60–80  $\mu$  wide, circular, entire, *pure white*.

*Spores* 6–7.5  $\times$  2–2.5  $\mu$ , *white, subcylindric*, slightly curved, ends obtuse or subacute, smooth, thin-walled, contents hyaline or faintly vacuolate, I -ve.

*Basidia* 9–13  $\times$  3–4  $\mu$  distally, 4–5  $\mu$  at the base, subventricose, with 4 slightly curved sterigmata 2.5–3  $\mu$  long.

*Cystidia* none.

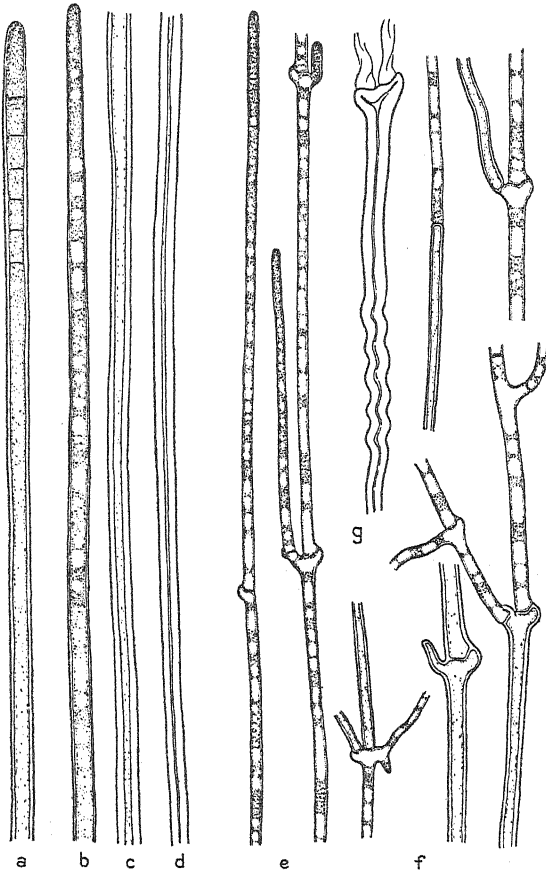
#### *The Hyphal Systems.*

Four kinds of hypha which differ in thickness of wall, in manner of septation or branching, or in size, are involved in the construction of the fruit-body, and each kind forms a system distinguished not so much by a common origin of the constituent hyphae, which need not be the case, as by a common function; a fifth system must therefore be added in the mycelial hyphae which connect the basidiocarp with the food-supply in the substratum.

*Skeletal hyphae*: thick-walled, unbranched, aseptate, straight or slightly flexuous, longitudinal, 2–5  $\mu$  wide, with the lumen more or less obliterated in mature parts, but the apices thin-walled with dense contents.

The skeletal hyphae present three kinds of apex. Those in the growing-point of the primordium, before the pileus has developed, taper gradually for 100  $\mu$  or so to a subacute apex, 2–2.5  $\mu$  wide (Text-fig. 1 *b*), and behind the apex the wall thickens very gradually, appearing as just a stout line under a  $\frac{1}{12}$ th objective at 30  $\mu$  back, and 0.2–0.4  $\mu$  thick at 70–100  $\mu$  back and 0.6–1.2  $\mu$  at 2–2.5 mm. back; and, as the hypha widens and the wall thickens, the protoplasmic contents become more and more attenuate. On the other hand, at the margin of the pileus the apices are 3–4  $\mu$  wide, broad and obtuse, and they do not taper. The wall begins to thicken about 5–10  $\mu$  back and thickens more rapidly than in the tapering kind: the apices appear of less vigorous growth. But whereas some of the apices are aseptate, many have 3–7 septa which are spaced at intervals varying from 4–12  $\mu$  apart (Text-fig. 1 *a*). The septa are slightly convex towards the apex and very thin. They are limited to the terminal 50–70  $\mu$

of the hypha and must therefore be reabsorbed soon after precipitation. Thus the one or two septa situated in the rear are mostly very thin indeed, and the last of all may be barely discernible as an excessively fine line and which represents perhaps just a belt round the lumen.

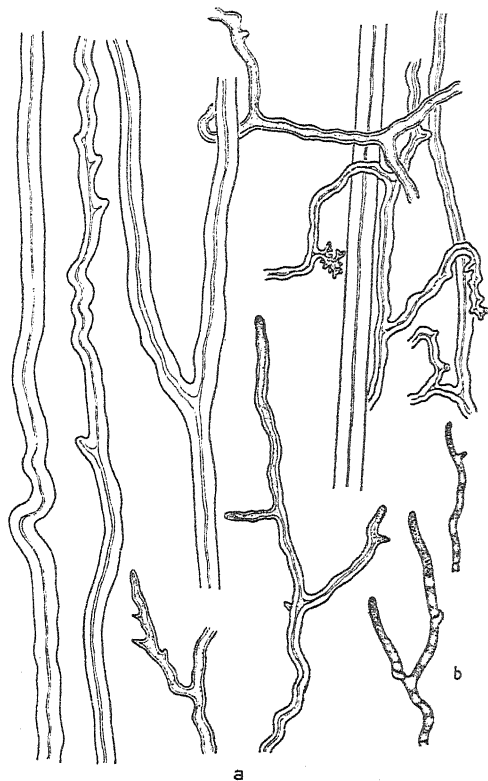


TEXT-FIG. 1. *a*, the septate apex of a skeletal hypha from the pileus; *b*, *c*, *d*, parts of a skeletal hypha from the primordial shaft, *b*, the apex, *c*, about 300  $\mu$  back; *d*, about 900  $\mu$  back; *e*, two consecutive parts of a generative hypha from the pileus; *f*, fragments of five generative hyphae from the pileus showing the origin of mediate hyphae; *g*, a fragment of a thick-walled generative hypha from the mature stem.  $\times 750$ .

*Generative hyphae*: thin-walled, branched, septate, longitudinal or interwoven, 1.5–2.5  $\mu$  wide, rarely 3  $\mu$ , with a clamp at each septum and abundant protoplasmic contents throughout, the cells 30–180  $\mu$  long with 0–4 branches from the distal end, occasionally from the middle or proximal end; H-connexions scarce (Text-fig. 1 *e*).

*Binding hyphae*: thick-walled, much branched, aseptate, interwoven, narrow, 1–2.5  $\mu$  wide, rarely 3  $\mu$ , with the lumen linear or obliterated in mature parts, often coralloid and flattened, kinked, nodulose or spiculi-

ferous, as if from mutual pressure and abortive branching, with the apices thin-walled or thick-walled, aseptate, and with scant contents in mature parts like the skeletal hyphae; H-connexions scarce (Text-figs. 2 and 3).



TEXT-FIG. 2. Fragments of four skeletal hyphae with a few binding hyphae from the mature stem: *a*, the apices of binding hyphae from the primordial shaft; *b*, the apices of narrow generative hyphae from the pore-field.  $\times 750$ .

*Mediate hyphae*: slightly thick-walled, sparingly or frequently branched, aseptate, flexuous, longitudinal or somewhat interwoven,  $1.5-3 \mu$  wide, with the walls  $0.5-1 \mu$  thick, with scant contents like the skeletal hyphae and the apices thin-walled and aseptate.

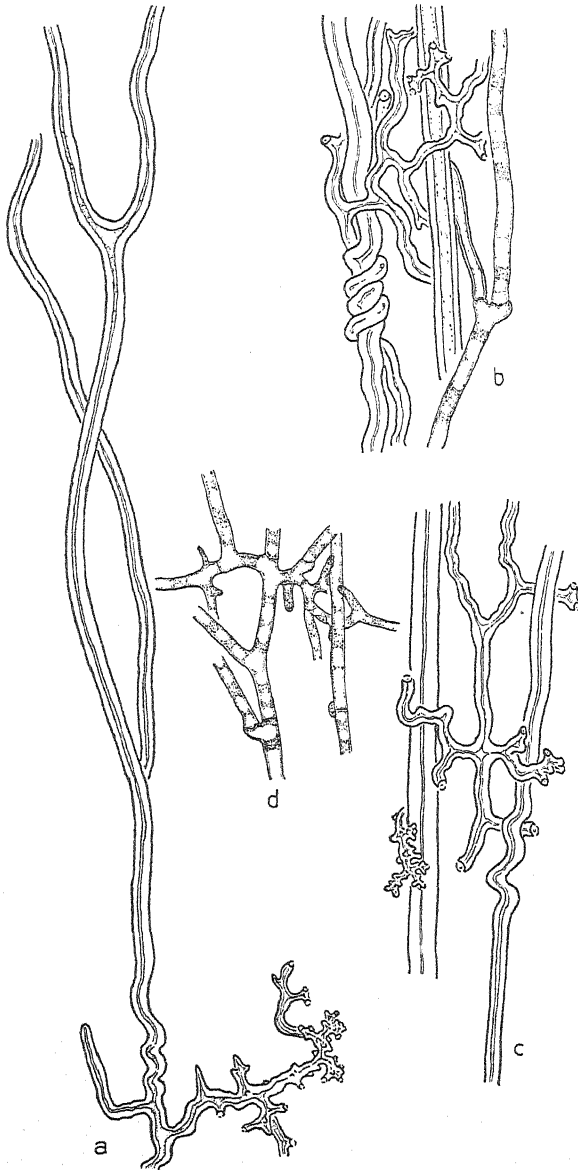
*Mycelial hyphae*: thin-walled or fairly thick-walled, branched, narrow,  $1-1.5 \mu$  wide; the thin-walled hyphae septate, with clamps, the thick-walled apparently aseptate.

#### *The Structure of the Basidiocarp.*

*The stem.* There are three concentric layers in the stem:

1. A thin superficial yellow or brownish crust,  $20-30 \mu$  thick, rarely so much as  $70 \mu$ , formed of binding hyphae with yellowish agglutinated walls

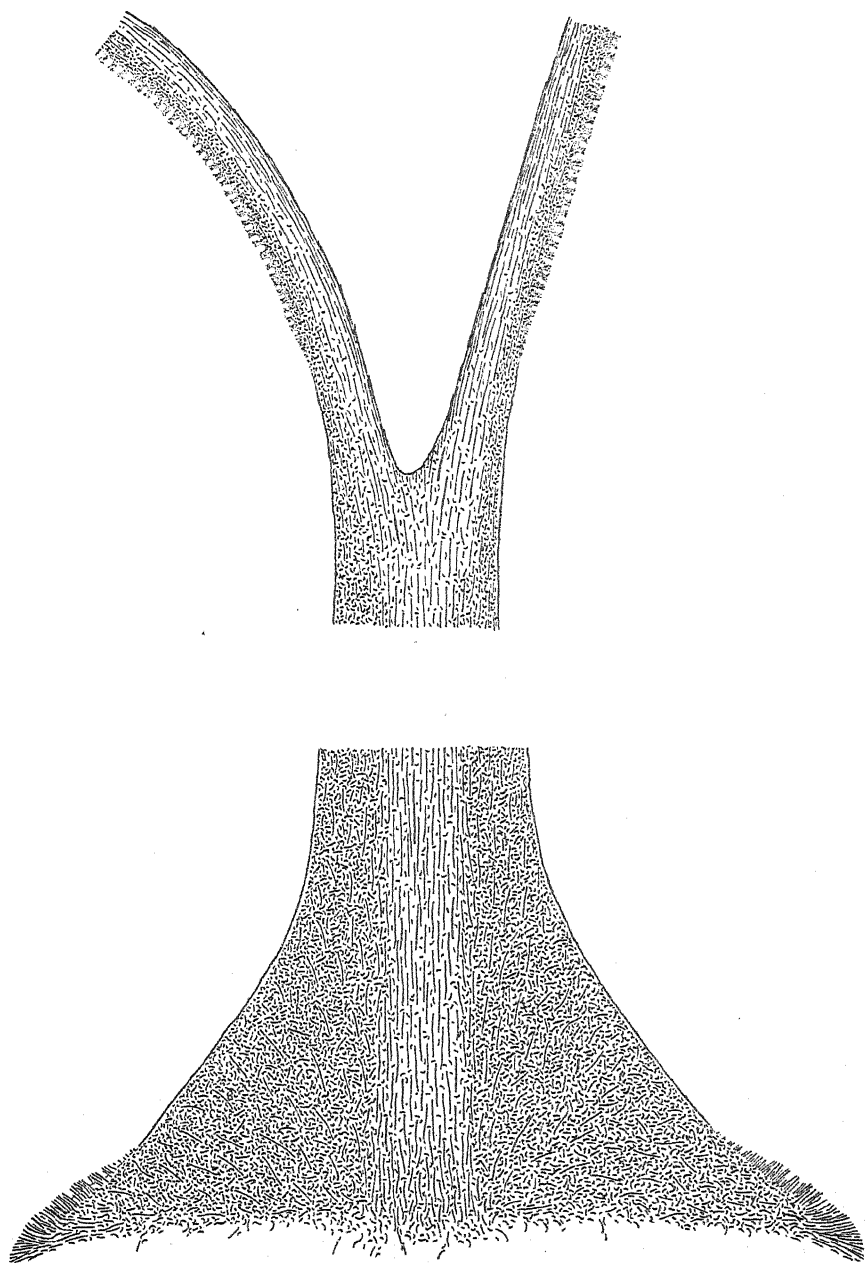
and of embedded portions of skeletal hyphae. The mature crust is an



TEXT-FIG. 3. *a*, part of mediate hypha which has given rise to a binding hypha; *b*, *c*, fragments of skeletal hyphae with binding hyphae; *d*, a knot of generative hyphae from near the apex of the primordial shaft.  $\times 1,000$ .

almost structureless resinaceous lamella in which the only sign of the original tissue may be traces of the narrow lumina.

2. A broad cylinder, 500–1500  $\mu$  wide, of longitudinal and closely



TEXT-FIG. 4. A diagram of the structure of the fruit-body : the disc and the base of the stem, the apex of the stem and the centre of the pileus are included : the diagram shows the distribution of the binding system, the superficial crust, the 'velvet', and the central core in the stem : the direction of the lines indicates the general trend of the hyphae.

interwoven hyphae, being the hyphae of the binding system spun so closely on the skeletal hyphae that the tissue is nearly solid. It merges into the superficial crust by agglutination of the walls of the outer hyphae.

3. A narrow central core of skeletal hyphae, longitudinal, with few binding hyphae, so that the tissue is loose, fibrillar, and readily teased asunder. Generative hyphae, chiefly longitudinal with a few narrow interwoven branches, occur in the core as well as in the intermediate cylinder, even in old stems, where, however, they are not numerous and they can be detected with certainty only by means of a cytoplasmic stain such as safranin.

*The disc.* The same three layers occur. The core is of the same width as in the stem, but the intermediate cylinder of very dense tissue is thickened to form the body of the disc. The yellow crust covers the surface except at the margin, where it is interrupted by a pile of hairs to form a very characteristic and specific feature which, from its 'plush' appearance, may be called the 'velvet'. The hairs project at right angles from the surface and are of all lengths up to  $450\mu$  (Text-fig. 5). They are  $4-5\mu$  wide but often slightly and irregularly swollen up to  $7\mu$  wide towards the base, tapering gradually to an obtuse or subacute apex,  $2-3\mu$  wide, thick-walled, and with the lumen more or less obliterated except at the free ends of the growing hairs, where the wall is unthickened; they are colourless, aseptate, unbranched, and slightly flexuous. The basal parts of the hairs, for a length of  $120\mu$  or so, are stuck together by the same crust as covers the stem, and the remains of binding hyphae occur between the hairs in this region. The extreme margin of the disc is a palisade or fringe of similar but decumbent hairs closely applied to the wood.

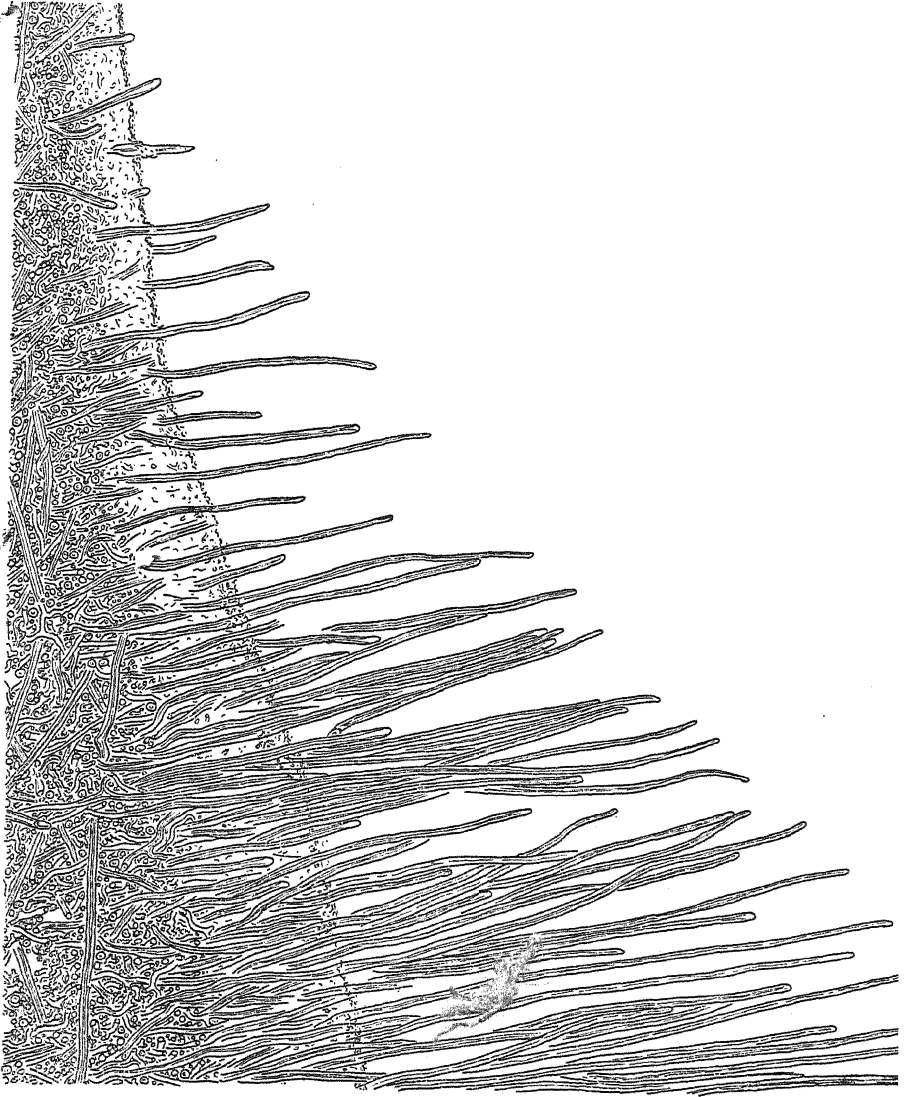
A few secondary mycelial hyphae grow from the under side of the disc, but the main connexion between the basidiocarp and the substratum lies below the central core.

*The pileus.* Three thin layers or plates of tissue, superposed and issuing from the apex of the stem, form the pileus:

1. A narrow layer on the upper surface, about  $20-30\mu$  thick, of longitudinal adpressed skeletal hyphae with pale yellow or pale brown, firmly agglutinated walls. The ends of the hyphae are simple, obtuse, thick-walled, and decumbent. A few generative hyphae occur even in the mature central parts of the pileus, but binding hyphae are absent. The layer is a surface-modification of the skeletal framework corresponding to the crust on the stem, although the constituent hyphae are continuous with those of the central core in the stem. The colour of the pileus comes from the pale yellow-brown walls of these hyphae and from a fuscous, yellow-brown or olivaceous pigment in the form of droplets in the narrow lumen. The pigment does not occur in the hyphae of the stem and hence the dark tawny-yellow of the pileus.



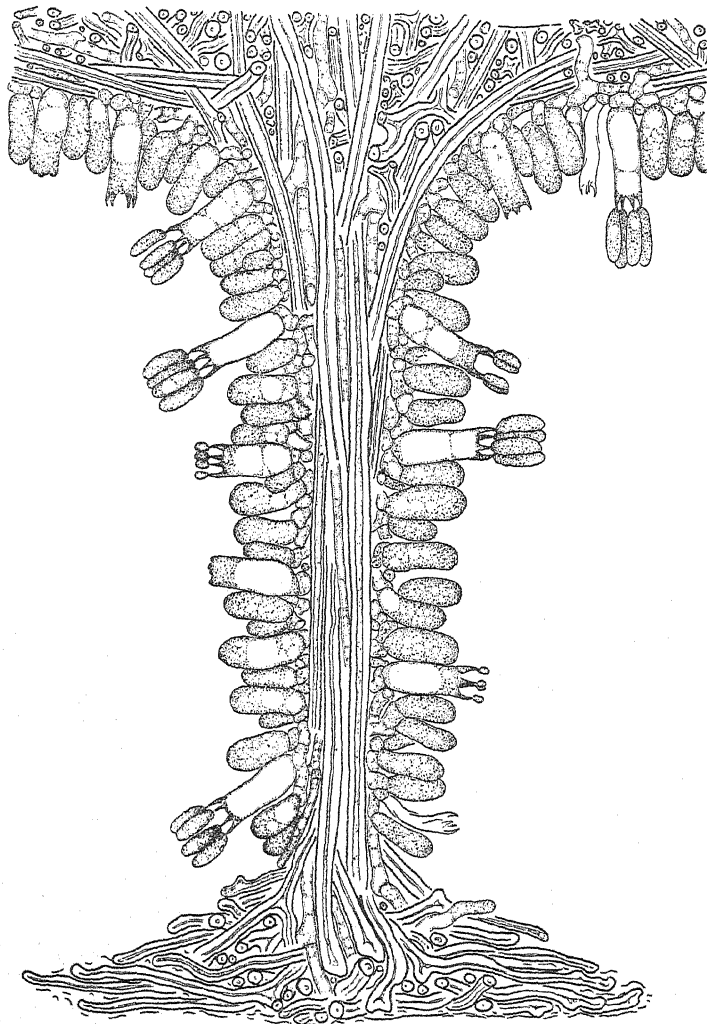
2. A wide middle layer, about 300–400  $\mu$  thick, of longitudinal skeletal and generative hyphae, with the skeletal elements predominant in the



TEXT-FIG. 5. A longitudinal section through the disc at the boundary of the 'velvet'.  $\times 250$ .

mature tissue. Binding hyphae are abundant in the lower part of the layer and stretch upward between the longitudinal hyphae singly or as scattered nests of coralloid branches. The tissue is mainly fibrillar and can readily be teased asunder. Mediate hyphae are especially abundant near the

margin of the pileus. This layer forms the body of the pileus and is continuous with the central core and inner part of the intermediate layer in the stem.



TEXT-FIG. 6. A longitudinal section through a mature dissepiment.  $\times 1,000$ .

3. A rather narrow layer,  $100-150 \mu$  thick, overlaying the tubes, composed of scattered longitudinal skeletal and generative hyphae very firmly felted by a mass of binding hyphae and narrow interwoven generative hyphae. Like the outer part of the intermediate layer in the stem, of which it is a continuation, this tissue is nearly solid and too dense to be teased apart except into small chunks with frayed edges. The branching

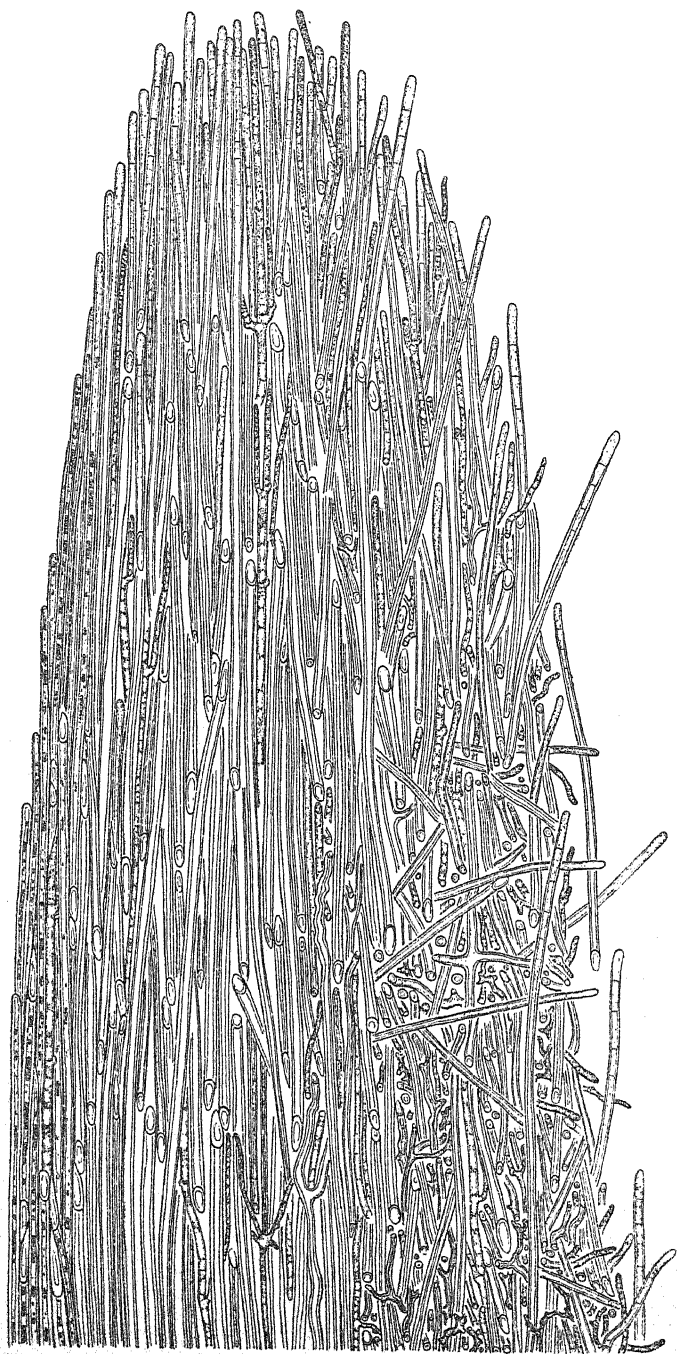
of the binding hyphae is exceedingly intricate, as if of the intention to fill every space between the hyphae of the skeletal framework. Beside a sinuous, inserting mode of growth, nooks and crannies are occluded by an exuberance of short processes and bosses, and in places both skeletal and binding hyphae themselves are flattened, kinked, or otherwise distorted from mutual pressure. It is remarkable that the tender generative hyphae are not crushed out of existence, yet they appear fully able to maintain a precarious position. The firmness and solidarity of this layer make the coriaceous texture of the pileus.

The crust of the stem stops at the apex, or it may be continued for 1–10 mm., equally or unequally, on the under side of the pileus. It gives place shortly and abruptly to the tubes; the superficial hyphae do not become agglutinated, and are left free to grow out from the surface.

*The tubes.* The dissepiments are composed of rather narrow longitudinal skeletal hyphae with a few generative hyphae, but there are no binding hyphae. The skeletal hyphae pass from the lower layer of the pileus vertically downward for 120–140  $\mu$  to the end of the dissepiment, where their apices may be lobed or shortly branched or deflected to one side and interwoven so as partly to occlude the pore-mouth (Text-fig. 6). The dissepiments are thus somewhat peltate in longitudinal section. The trama of longitudinal hyphae varies from 5–15  $\mu$  wide, and in places may be only one or two hyphae thick. The hymenium is continuous over the dissepiments down to the flange at the mouth. The basidia are borne on a very thin layer, 1–2 hyphae thick, of narrow, much branched, and short-celled generative hyphae, 1–2.5  $\mu$  wide, which may have come directly off the longitudinal generative hyphae in the trama or from those in the lower layer of the flesh.

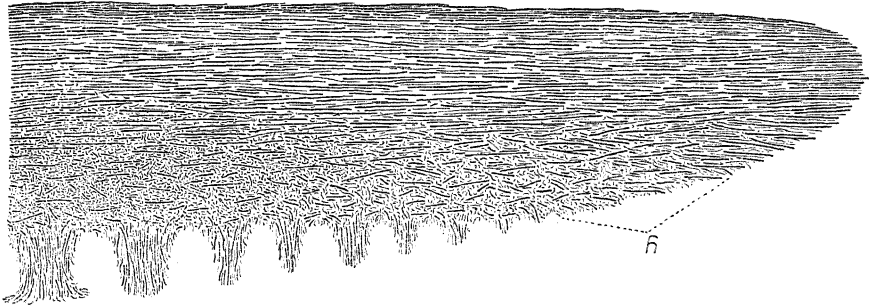
#### *The Marginal Growth of the Pileus.*

A sheaf of more or less parallel but intertwined skeletal, generative, and mediate hyphae form the margin of the pileus. In radial section the margin has a slight conical outline indicating that radial, or marginal, growth is greatest in the middle of the sheaf and slacks off above and below. The tips of these hyphae, dressed compactly to a common front, form a naked, unprotected growing-point. They are directed obliquely outward from the apex of the stem, eventually becoming horizontal, and by centrifugal growth they build the funnel-shaped limb of the pileus. On the upper side, about 500–1000  $\mu$  behind the margin, the skeletal hyphae are agglutinated into the resistant surface-layer and the fuscous pigment develops in the hyphal lumen, but on the lower side they are looser, often projecting up to 50  $\mu$  or so, and commingled with many mediate and generative hyphae. The binding system pursues some 200–300  $\mu$  in the rear through the lower part of the pileus. It spreads over the skeletal framework as a fan-plate, the



TEXT-FIG. 7. A longitudinal section of the margin of the pileus.  $\times 350$ .

advancing front of which is about  $30-40\ \mu$  high, but it deepens gradually to  $100-150\ \mu$  by the profuse interstitial branching and insinuation of its hyphae between the longitudinal elements, thrusting them apart and consolidating the framework, and freely also by downgrowth on the under side. The



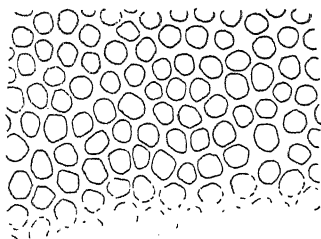
TEXT-FIG. 8. A diagram of the structure of the pileal margin : *p*, the pore-field.

binding hyphae penetrate upward to some extent also into the main mass of tissue, but in numbers insufficient to affect the fibrillar texture, and they do not reach the surface-layer. At  $2-3\ \text{mm.}$  from the margin the tissue is complete.

### *The Development of the Tubes.*

The great activity of the binding hyphae occurs in a region stretching from  $300-1500\ \mu$  from the margin, though it may be only half so broad, and which appears round the periphery on the under side of the pileus as a pure white, even, velutinate zone. The binding hyphae are accompanied by many narrow, downgrowing generative hyphae,  $1-2\ \mu$  wide ; they do not take a direct course but ramify and deviate in a senseless manner to form an interwoven tissue, not a palisade, behind them. This zone of even growth may be called the *pore-field*, because here play the lines of force which demarcate the pores ; it is in a part highly charged with vitality that the characteristic of the polypore takes expression. At the back of the field the pores are delimited at full size as subangular and roughly hexagonal areas of restricted growth girt by excrescent ridges, and these ridges, advancing radially *pari passu* with the margin of the pileus, branch and unite round the pore-patches, fashioning the honeycomb. The hyphae which form the ridges are altered in such a way that very shortly after the excrescence has begun the binding hyphae, through a slight enlargement of the apex and loss of branching—and, in addition, many of the generative hyphae, by thickening of the wall and loss of septation—are transformed into skeletal hyphae which continue the downgrowth to form the dissepiments ;

the evidence for this remarkable transition will be discussed presently.



TEXT-FIG. 9. A part of the pore-field.  $\times$  ca. 25.

Thus the framework of the tubes is built in much the same manner as that of the pileus.

About 2–3 mm. from the margin the downgrowth stops, and the dissepiments are completed by the partial occlusion of the pore-mouths. The immature dissepiments appear scarious owing to their thin edges, and the pores subangular, and the transition to the smooth round orifice at maturity is quite abrupt.

The hymenium is formed from narrow, 1–2  $\mu$  wide, flexuous generative hyphae which project into the developing tubes. They branch and descend the dissepiments, receiving laterals from some of the generative hyphae in the trama. They project roughly at right angles from the trama and the apical cells

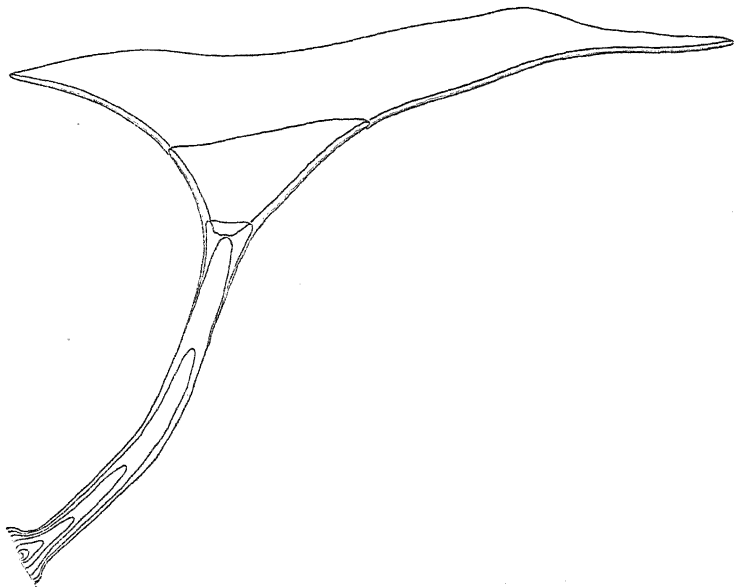
enlarge into basidia, after which other basidia are intercalated sympodially from the subapical cells to form the continuous hymenium: no apical cell is sterile.

### *The Development of the Primordium.*

The primordium appears on the surface of the wood as a minute white fleck which enlarges into a little hemispherical cushion, some 500–1,000  $\mu$  wide. A vigorous growth soon begins over the centre of the distal side and the primordium elongates, becoming conical, and a shaft varying from 4–50 mm. in length is eventually laid down. The hyphae in the central part of the apex then stop growing, while those at the periphery continue to construct the pileus: so the apex flattens, becomes narrowly cyathiform, and passes over into the funnel-shape of the pileus. The length of the shaft, as well as of the pileus, is due solely to apical growth of the hyphae; there is no subsequent elongation, and, therefore, no motor-tissue. The shaft expands behind the apex through the development of the binding system until the width of the mature stem is reached, and especially at the base does it dilate to form the disc, completed generally before the pileus has appeared. A main centre of growth at the apex of the primordium and a subsidiary one at the base thus produce the single axis with terminal pileus and discoid foundation.

In two primordia which developed on separate sticks under a bell-jar, apical growth stopped after a shaft about 30 mm. long had been produced, except at two points on the periphery in one case and three in the other.

From each point grew a new shaft, and in each primordium one of these secondary shafts, after reaching a length of 5–6 mm., bore a pileus 15 mm. wide, whereas the other secondary shafts reached only 3–4 mm. and pro-



TEXT-FIG. 10. Successive stages in the development of the fruit-body in longitudinal section and orientated in natural position with respect to the vertical.  $\times 2$ .

duced only abortive pilei, 1–3 mm. wide. I have not met with other instances of branching in this species, though occasionally wild specimens may be found with one or two small pilei which have proliferated from the centre of the upper side.

In certain places in the superficial vessels of the wood the mycelial hyphae form a close skein, and from thence project the subaerial hyphae of the primordium. The hyphae in the very young primordia, less than  $300\ \mu$  high, cannot be traced far enough to disclose their origin because they are too densely interwoven, and these very small bodies are difficult to section. But in no case did I find on teasing asunder primordia of  $100$ – $200\ \mu$  high that they were composed of only one kind of hypha, but of all kinds. Skeletal, generative, and binding systems must therefore each be present at the start as direct products of the mycelium.

At first the hyphae are interwoven, and grow in any direction at the surface of the primordium. In a day or two they come into alinement over the central part to form the growing-point of the primordial shaft and they continue parallel, though intertwining, in a sheaf until marginal growth of the pileus is arrested, generally some 50–120 mm. distant from the

substratum. Skeletal and generative hyphae, in roughly equal numbers, with few mediate hyphae, compose the growing-point and, as described already, the apices of the skeletal hyphae are tapering, narrow, aseptate, and indistinguishable from those of the generative hyphae, which lie juxtaposed and intermingled.

The binding system follows at some distance from the apex and towards the outside. It develops in two ways, indirectly from laterals of the generative hyphae some  $500\ \mu$  behind the apex, which branch freely and become thick-walled and aseptate, and directly from the upward creep of the binding hyphae from the base of the primordium. The hyphae intrude between the longitudinal elements and branch very freely, just as in the pileus, and the consolidation of the fibrillar stroma begins on the outside about  $500\ \mu$  behind the apex of the shaft and proceeds gradually inward, the central core of the mature stem being the remnant of the original stroma, but imperfectly bound: even in primordia in which the pileus has just begun to develop the distal portion of the stem is still mainly fibrillar. Thus the shaft thickens, and at the base the disc forms by a vigorous outgrowth of binding and narrow generative hyphae. The apices of these hyphae do not take a straight course, but interweave to form the body of the disc and the generative hyphae are turned eventually into binding hyphae. When the disc is nearly full-sized the apices of many of the hyphae at the periphery enlarge to a width of  $5\text{--}7\ \mu$ , and grow out more or less straight for about  $500\ \mu$ : the apices, when growing, are thin-walled, aseptate, obtuse, and slightly tapering, and the wall thickens close behind. Other binding hyphae, with the usual tortuous growth, penetrate between the bases of these excrescent elements and fasten them together, but, as they do not approach within  $300\text{--}400\ \mu$  of their extremities, the distal portions remain free to form the pile of the velvet.

The crust develops from the base of the stem upward to the pileus and outward to the edge of the disc. It may begin when the primordial shaft is only half grown, or not until the pileus has appeared, and its formation is marked by the turning of the white, velutinate, immature surface to a smooth cream-colour which, in turn, may darken in blotches to an ochraceous yellow; rarely does the whole surface become yellow-brown. The walls of the superficial hyphae, which belong mostly to the binding system, and thus are often very closely, intricately, and shortly branched in a coralloid manner, but which include also the ends of a few narrow generative hyphae and the shanks of outlying skeletal hyphae, become agglutinated and yellowish, and finally a glassy resinaceous layer faintly mottled by the narrow lumina of the hyphae is all that remains of the original structure: the layer is probably dead by the time the fruit-body has matured.

In many cases very shallow pores develop round the primordial shaft

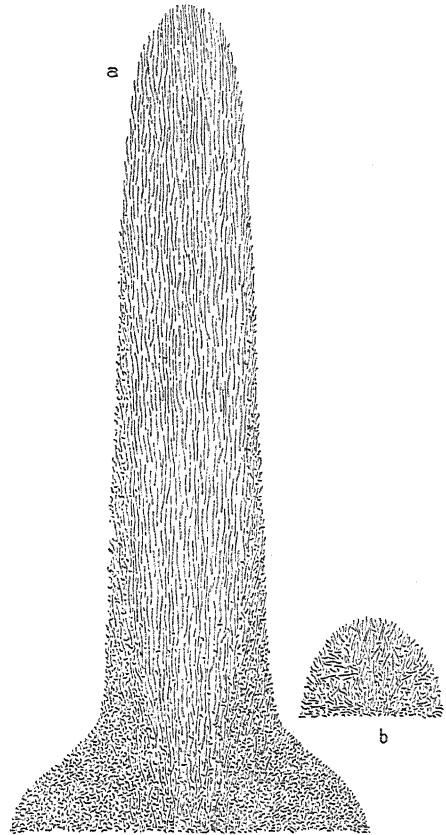


in a belt which is 2–3 mm. wide and situated 1–2.5 mm. from the apex, and as the shaft lengthens so the pores are occluded from below upwards; a pore-zone moves along the shaft, and not until the apex dilates into the pileus does it become stabilized.

The dissepiments are formed as on the pileus from excrescent binding and generative hyphae, and sometimes they are fertile bearing scattered basidia, but the pores themselves are soon filled in with other binding hyphae and eventually the surface hardens into the yellow crust. The pores may appear on primordia only 5 mm. high, which subsequently produce a stem 6–20 mm. long, or not until the stem is almost full length; and if, for some reason, the growth of the shaft is prematurely arrested, the pores may develop to the apex and the distal portion is thus covered with a shallow honeycomb. On the other hand, pore-formation may be delayed for 1–10 mm. along the under side of the pileus, in which case the crust of the stem spreads on to the pileus. But a wholly poreless, pileate specimen I have not seen; though, considering how restricted is the growth of the tubes in this fungus, such a stereoid form might be predicted: it would be interesting to know how *Stereum curtum*, Fr., compares

in structure, because Petch mentions that some abnormal specimens of a *Stereum*, which he refers to *S. curtum*, bear a marked resemblance to *P. xanthopus* (10). The crust gives place abruptly to the tubes, but not necessarily at the same level all round the stem, so that the limit of the crust is a sinuous line, and there may even be small islands of crust, at 1–2 mm. from the stem-apex, surrounded by pores.

Marginal growth of the pileus is at first regular, and if the primordium has grown vertically from a horizontal surface, it so continues to give a centric pileus. However, the primordium as often grows from a vertical



TEXT-FIG. 11. A diagram of the structure of the primordial shaft at two stages of development: *a*, from a specimen 6 mm. high,  $\times 20$ ; *b*, from a specimen 350  $\mu$  high,  $\times 40$ : the disc is still enlarging, and in neither has the superficial crust yet appeared.

or oblique surface and the shaft is upcurved, in which case marginal growth is soon arrested on the incurved side next to the wood and, as the remainder spreads fanwise, an excentric pileus results, with a hilum more or less strongly marked according to the extent of inhibition on the inside. Rarely is the pileus reduced on the inside to a mere rim, 1–2 mm. wide, as when the shaft has grown nearly horizontally. And the basidiocarp is never truly spathulate or flabelliform, i.e., with a stem which dilates unilaterally into the pileus and without a trace of limb to the inside. In large specimens marginal growth finally becomes irregular, probably on account of the difficulty of feeding uniformly so diffuse a meristem, and thus, as growth is arrested in some places, the margin becomes sinuous or lobed.

### *The Interrelation of the Hyphal Systems.*

The margin of the pileus grows centrifugally and maintains an even front of almost constant thickness. The number of hyphae at the margin must therefore increase, doubling as the radius of the pileus doubles, yet the margin is composed primarily of skeletal hyphae which do not branch. Of all the hyphal systems in the primordium the one which cannot multiply its own members builds the expanding framework of the pileus. Some other system must produce skeletal hyphae. Moreover, at an early period of development, when the apex of the primordial shaft widens from  $200\ \mu$  to  $1,000$  or  $1,500\ \mu$ , the skeletal hyphae must increase greatly in numbers. Also, the cessation of growth of the hyphae on the outside of the growing-point in both the primordial shaft and the pileus inflicts a continual loss on the skeletal system which must be repaired. There is little doubt that the generative system provides the skeletal hyphae, yet not by bodily transformation of its own members, since that would imply thick-walled, septate, and branched hyphae, but indirectly from laterals,  $1.5$ – $2.5\ \mu$  wide, the apices of which gradually expand to  $3$ – $4\ \mu$ , while the wall begins to thicken posteriorly and they lose the power of septation with concomitant clamp-connexion. Such laterals are often to be found connected with generative hyphae in the neighbourhood of the growing-point (Text-fig. 1 f). They are the mediate hyphae. They branch rather sparingly, the branches growing longitudinally, and both parent and branch must eventually become skeletal elements. I have never traced a skeletal hypha to a generative hypha, however, presumably because the distance involved exceeds the lengths of hyphae which can be teased without breaking from thin sections. But I have traced skeletal hyphae for as much as  $1,000\ \mu$  from the apex and parts of others for the same distance and frequently seen that they narrow from  $3$ – $4\ \mu$  distally to  $1.5$ – $2.5\ \mu$  proximally, where they may be somewhat flexuous and have all the appearance of mediate hyphae, to an occasional branch even (Text-fig. 2). And mediate hyphae I have

traced from generative hyphae for as much as  $500\ \mu$ , and seen that they enlarge to  $3\text{--}3.5\ \mu$  and straighten. The suggestive train of intermediates occurs in the growing region from 1–4 mm. back from the margin of the pileus or at the apex of the primordial shaft.

The skeletal hyphae in the dissepiments differ in origin from those of the stem and pileus. They are derived from binding hyphae which have grown out in ridges in the pore-field as already mentioned. They can be traced to the lower layer of the pileus, where they narrow to  $1.5\text{--}2\ \mu$  wide and become sinuous and not infrequently branched, and they are shortly lost in the maze of interwoven hyphae; and many intermediate stages in the widening and straightening of the binding hyphae can be seen in the young ridges. Moreover, they cannot be derived from the skeletal system of the pileus, because in that case a few of those hyphae, at least, must be deflected through nearly  $90^\circ$ , or they must branch, and neither such bending nor branching has been seen. Likewise at the periphery of the disc the binding hyphae, in vigorous excrescence to become the hairs of the velvet, assume all the properties of the skeletal hypha. Many of the generative hyphae which are incorporated in the downgrowing ridges must also change into skeletal hyphae, because generative hyphae are much more abundant in the young ridges than in the mature dissepiments, and there is a corresponding increase in the numbers of skeletal elements. These generative hyphae apparently become aseptate shortly after emergence from the tissue of the pileus, but the wall does not begin to thicken until they are nearly of full length.

The binding system, on the other hand, is self-contained. It receives contributions, nevertheless, from both mediate and generative systems. The mediate hyphae, shortly after their origin from generative hyphae, bear laterals at a wide angle, and these laterals, having no sense of direction, but a great power of ramification, push vaguely into all free spaces and become binding hyphae (Text-fig. 3 a). Narrow laterals from the generative hyphae, especially near the growing-point of the primordial shaft, may also become binding hyphae directly by loss of septation and by thickening of the wall.

The mediate hyphae, being a developmental phase, perhaps scarcely form a system comparable with the others. Yet, as the basidiocarp is virtually of unlimited growth, they are a constant feature of the marginal meristem and, in the mature tissue, they make the connexions between the elements of the other systems, with none of which can they strictly be classified. They differ from generative hyphae, from which they always arise, in being thick-walled and aseptate, from skeletal hyphae in being branched and narrower, and from binding hyphae in being much less branched and longitudinal. They are a go-between, and it is preferable to hold them apart.

The generative hyphae are the vital reproductive elements. As occasion demands, they can turn out the hyphae of any other system; they form eventually the hymenium; and they provide probably the main channel of nutrition from the mycelium. The walls of many remain thin throughout, and the cytoplasmic contents fairly dense, but in others, perhaps with senescence, the walls are heavily indurated and the contents largely vacuolate; for what are clearly fragments of thickened generative hyphae, with a terminal unilateral bulge to indicate the thickened clamp, were often found on teasing out the tissue of the old stem (Text-fig. g).

### *Problems of Development.*

In the higher fungi, whether ascomycete or basidiomycete, the same problems recur in studying the development of the fruit-body. The subject is properly one for experiment, but it must first be approached through microscopical investigation to learn in what way the living matter grows. It is worth while to press farther into the form-factors and anatomical peculiarities of this fungus, if only to realize what a deal of organization is required even when development appears simple and direct. For it is yet a marvel how a mycelium, which is buried in the substratum, can send into the air hyphae without experience to build instinctively, so deftly and unerringly, a shapely structure of specific form: it is a problem, really, of the past.

The form-factors may be considered separately, and roughly in order of development, though in reality no such distinctions can be made.

1. *The inception of the fruit-body.* The prime factors in the presence of which the hyphae grow out from the wood into the air are the accumulation of food-material by the mycelium, and abundant moisture in the substratum to the extent of saturation. But perhaps there should be added a temporary or permanent exhaustion of the food-supply in the substratum. It has been shown experimentally in several instances that an artificial check to mycelial growth, when the food-supply is plenteous, will provoke the development of the fruit-bodies. Wakefield (13) obtained fruit-bodies of *Stereum purpureum* and *Schizophyllum commune* in culture in this way on reducing the supply of food, and others have employed the inhibitory action of strong light. Bose has recently discussed the relation at length (2), but apparently it does not always hold (1). Moreover it is obvious that the mycelium cannot give rise to a fruit-body, especially if it is in the nature of the species to produce large fruit-bodies, without preliminary acquisition of food, and the substratum must needs be exhausted to a greater or lesser extent where the fruit-body will develop.

The emergent hyphae may be phototropic. Light is required for the development of the fruit-body of *Polyporus pergamenus* according to Rhoads (11), of *Lenzites malaccensis* according to Bose (2), and for the

development of the primordia of many of the species of Polyporus, Fomes, Daedalea, Trametes, &c., studied in culture by Harsch and Long (8), but not in the case of *P. Farlowii*, *P. cinnabarinus*, or *Trametes serialis* studied by the same authors, nor for the development of the primordium of *P. squamosus* according to Buller (3). There are also records of naturally occurring polyporoid growths made in utter darkness, as the case of *Fomes ulmarius* in a London sewer (12). On the other hand, it seems to be a demand for better means of respiration, such as the large amount of oxygen or small amount of carbon-dioxide in the atmosphere should afford, which leads the hyphae into the open. Besides being aphotic the conditions in the substratum must be nearly anaerobic, and the hyphae will surely respond to a pressure of oxygen many times greater than that to which they have grown accustomed. There is a point in the artificial culture of polypores which colours this suggestion. It is remarked (1, 2, 8) that primordia, however abortive, generally develop at the upper end of the agar-slant or round the edge of a Petri-dish. These are loci which correspond with the optimum positions for aeration which the circumstances allow. In the case of *P. hispidus* Baxter proved conclusively that such a tendency was not connected with the drying-out of the agar, nor with depletion of the food-material (1). Accordingly one may suppose that by improving the means of aeration, commonly left to the diffusion of oxygen from the atmosphere through a tight plug of cotton-wool, there would be a better chance of obtaining the fruit-bodies of some polypores in artificial culture, though one can scarcely expect the big fruit-bodies of *Ganoderma applanatum*, *P. hispidus*, or the like to develop normally in such cramped surroundings. But both these difficulties Etter (14) has recently overcome very successfully and ingeniously, the one by adding nutriment, in the form of malt liquor, to the primordium at critical stages in development, and the other by allowing the growing pileus itself to push out the plug of cotton-wool from the neck of the flask and, thus projecting, to complete development in the open air. By these means characteristic and full-sized fruit-bodies of *Lentinus lepideus*, *Pleurotus ostreatus*, and several wood-destroying polypores have been obtained.

The hyphae turn from the detachment of individual activity in the substratum and combine to form a tissue. The morphological character of the mycelium, if such it can be called, is the separation of the hyphae, which is partly the effect of outgrowth into regions of better food-supply, but chiefly an active avoidance of each other on account of the excretion of those 'staling substances' which are formed during the acquisition and synthesis of food-material. Now the hyphae of the fruit-body do not have to acquire food in this manner; it is supplied from the mycelium, and they probably do not produce staling substances. It seems therefore that, contrary to the immediate view, under the conditions of good oxygenation

and ready-made food-supply the hyphae are naturally concrescent, and that the lax, dissociate character of the mycelium is induced by want of oxygen in the substratum, by the foraging after food, and by the mutual repulsion arising from the formation of staling substances. The problem remains, nevertheless, why the hyphae should become irritable in this manner.

2. *The formation of the stem.* The emergent hyphae grow at first in all directions, forming a hemispherical cushion, but such uniform expansion gives place shortly to apical growth whereby the primordium becomes a cylindrical shaft. The establishment of apical growth may present the first actual limitation, i.e. by restriction of sideways growth, or it may merely be the result of deficient branching among the hyphae. If the primordium were to continue hemispherical the generative hyphae would need to branch very frequently to produce the required number of skeletal hyphae, because the number of outgrowing hyphae must be quadrupled when the distance from the point of origin is doubled, in accordance with the relation between the surface of a sphere and its radius. If the generative hyphae cannot effect this measure the primordium will assume a shape with less surface on account of concrescence of the hyphae; it will tend automatically to become cylindrical. And having become cylindrical the production of skeletal hyphae just counterbalances the loss of these elements by cessation of growth of those on the outside of the growing-point.

The initial direction of the shaft away from the substratum may be referred to the natural tendency of the hyphae to grow straight, to concrescence, and perhaps also to a repulsion from the carbon-dioxide accumulated on the surface of the wood. In a few days, however, the growing-point becomes positively phototropic. All the primordia which I raised in the laboratory responded to the unequal illumination from the windows: in one case a primordium which had arisen on the side of a branch away from the window turned through nearly  $180^\circ$  in the course of a week. Since there is no motor-tissue, such curvature of the shaft is due to an alteration in the direction of growth of the hyphal apices in the growing-point. I have seen no suggestion of geotropism in the behaviour of the primordial shaft.

3. *The formation of the pileus.* This can be resolved into five processes:—

- (a) The check to apical growth of the primordial shaft.
- (b) The peripheral expansion of the apex of the shaft to form the limb of the pileus.
- (c) The orientation of the limb.
- (d) The formation of the limb.
- (e) The excentricity of the pileus.

(a) The check to apical growth of the shaft is probably, in part at least, a photic stimulus. Buller found in the case of *Polyporus squamosus* that it was sufficient to expose the primordia to light for an hour only to induce the development of the pileus, and in the case of *Lentinus lepideus* that the primordial shaft dilated into the pileus only when the apex was acted on by light of a sufficient intensity.

Several of the first primordia of *P. xanthopus* which I raised produced pilei of 1–2 cm. diameter, but latterly, when intending to make some experiments, I was unable to obtain pileate specimens in my cultures. Thinking that they were too wet, I allowed them to dry out slowly under the bell-jar, but the primordia continued unaffected until the air became too dry and growth was suspended. I tried them in weak and strong daylight, but to no effect. In any case there must be two factors concerned in the initiation of the pileus, one of which is external and probably photic, and the other is internal and responsive; and presumably the internal mechanism was at fault in my specimens.

(b) The stimulus to the development of the pileus affects the apical growth of the primordium in two ways. It stops growth in the central part and increases the branching at the periphery. The apex of the shaft dilates and the direction of apical growth, which now becomes the marginal growth of the pileus, is displaced from alinement with the stem towards the horizontal. It is possible that the change of direction may be a deliberate motive on the part of the hyphae; on the other hand it can be explained on a physical basis. A great increase in branching at the apex of the shaft will cause the hyphae towards the periphery to be splayed aside in order to accommodate the new laterals, and the more so because the proximal parts of these hyphae are not only entwined with each other but firmly held by the binding system: in longitudinal section the apex of the shaft shows such a divergent sheaf of hyphae. And since the hyphae over the central part of the apex of the shaft have stopped growing, the main region of apical growth will be displaced towards the periphery, and the direction of apical growth towards the horizontal, on account of the deflection of the free ends of the peripheral hyphae. In fact it seems that the shape of the pileus in many basidiomycetes is determined by the two processes, the one of *expansion* or multiplication of the hyphae, and the other of the formation of a *dead space* or area over which apical growth is arrested. The point becomes clearer on considering the simplest ways of interaction of the two processes.

So long as there is neither expansion, nor the formation of a dead space, the primordial shaft remains cylindrical. But, if there is a stimulus which causes expansion, then the apex of the shaft dilates into an inverted cone with curved base to give a turbinate pileus (Text-fig. 12); or, if expansion is very great, then the pileus which results may be nearly

spherical, with the sides recurved against the stem. If there is no expansion but a dead space forms, then a tubular body results (Text-fig. 13); and this occurs in the pore-field in the generation of each tube. And if there

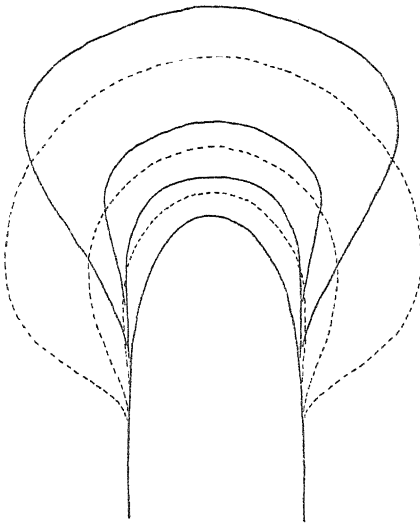


FIG. 12.

TEXT-FIG. 12. A diagram of the formation of a pileus by the process of expansion: the continuous line represents relatively slight expansion, the broken line relatively great expansion.

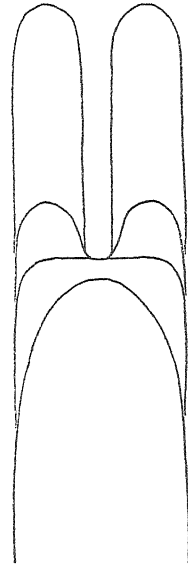


FIG. 13.

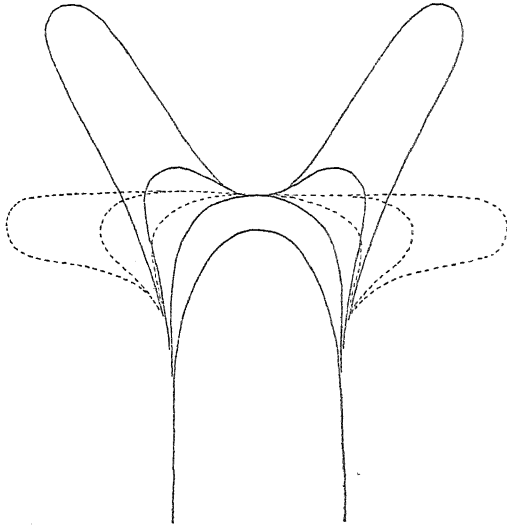
TEXT-FIG. 13. A diagram of the formation of a dead space, without expansion, at the apex of a primordial shaft.

is both expansion and a dead space, the pileus becomes obconic and more or less funnel-shaped (Text-fig. 14). The peltate pileus (Text-fig. 14) may result from great expansion and a large dead space, and conceivably the deconic pileus may result from excessive expansion accompanied by a very large dead space indeed which extends almost to the periphery: a maximum dead space, coincident with the whole apex, of course merely inhibits further outgrowth.

The effect of the dead space is to restrict the growing margin of the pileus to the periphery of the divergent sheaf of hyphae at the end of the primordial shaft, and in such a way that marginal growth of the pileus produces a limb the upper and lower sides of which are parallel or slightly convergent from the centre outwards. Thus great expansion and a small dead space would leave so wide a marginal growing-point that a limb with divergent sides would form, and this is apparently an impossibility. The size of the dead space is thus mainly determined by the degree of expansion, and the one defines the other. It should be remarked, moreover, that the



formation of a dead space refers only to the cessation of apical growth in the growing-point ; expansion can also take place within the dead space by sympodial branching of the hyphae, and it will cause a greater divergence in the direction of marginal growth of the pileus from that of the primordial shaft.

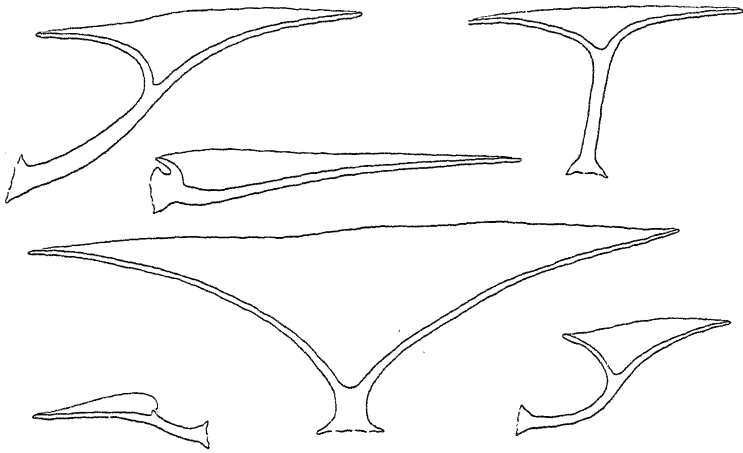


TEXT-FIG. 14. A diagram of the formation of a pileus by means of expansion and a dead space: the broken line represents the formation of a peltate pileus from a high degree of expansion and a very large dead space.

Neither cylindric, tubular, nor deconic pilei occur among polypores, and if ever the pileus took a turbinate shape the evolution of the dead space would have brought a great economy in building material.

(c) From the drawings of mature fruit-bodies shown in Text-figs. 10 and 15, it can be seen that the direction of marginal growth of the pileus inclines more and more to the horizontal, which, however, it never exceeds. Two factors, one physical and the other physiological, may be involved in the orientation of the limb. As the limb develops, the hyphae on the upper side are agglutinated into a crust. The crust acts like a stay and prevents the ingress of the margin into the obconic region defined by the dead space. For mechanical reasons, therefore, the inclination of the limb to the horizontal will not decrease. In the second place, both gravity and light may be directive. That the limb gradually becomes horizontal may be ascribed to a slow diaphototropic or a slow diageotropic response. In the case of *Polyporus squamosus*, Buller found that the margin of the pileus was definitely diageotropic; when the position of the pileus was altered from the horizontal, the margin grew in such a manner that the newly formed limb was again horizontal; and similar deformed fructifications

may be met with in nature when, for instance, on the fall of a dead trunk a new pileus develops at right angles from the first. But in the case of *P. squamosus* light is certainly ineffective; primordia exposed for a couple



TEXT-FIG. 15. Sections of variously shaped fruit-bodies, the primordial shafts of which have grown at different angles with the horizontal: the sections orientated naturally with respect to the vertical: nat. size.

of days to light, in order to induce the development of the pileus, subsequently grew normally, and became fertile in complete darkness.

(*d*) So soon as marginal growth begins, a centrifugal component is introduced which implies tangential growth of the pileus. The shape of the limb, as seen in radial longitudinal section, will depend on the ratio between centrifugal and tangential growth, i.e. on the ratio between apical growth and branching of the hyphae in the meristem. As the hyphae grow radially, they must diverge from each other. Spaces tend to appear between them, and the limb would thin off radially owing to concrescence of the hyphae were not sufficient branches produced to fill the spaces. Such centrifugal attenuation leads perhaps in the long run to fimbriate and ciliate margins where radial growth has outstripped tangential. It occurs sooner or later in all pilei, probably because the decline in the rate of food-supply, as the distance from the substratum increases, affects radial and tangential growth unequally.

Thus, taking the simplest case of a circular pileus with horizontal limb, on doubling the width of such a pileus the number of hyphae in the marginal meristem must be doubled if the margin is to remain at constant thickness; it is the relation of the radius of a circle to the circumference. If  $r$  be the initial radius, then on increasing to  $2r$ , by marginal growth, the skeletal, mediate, and generative hyphae will have all increased in length by  $r$ , but the mediate and generative hyphae will also have produced

between them as many laterals as there were separate hyphae in the initial meristem at radius  $r$ , and these branches will themselves have grown a considerable fraction of the distance  $r$ . In other words, if the margin is to remain of constant thickness, the mechanism for tangential growth must do almost double as much work in the same time as that for radial growth, and presumably it requires double as much fuel. Because a skeletal hypha, under these circumstances, can grow almost double as far radially as a mediate or generative hypha on the same amount of food, a decline in the rate of food-supply will most probably first retard tangential growth. Actually, however, when the limb is inclined to the horizontal, as in the young pilei, the disproportion between radial and tangential growth will be smaller, and it will diminish as the angle between the limb and the horizontal increases. So in old fruit-bodies with the limb nearly or quite horizontal, and the growing margin far removed from the substratum, the limb will assuredly be attenuate.

If the hyphae branch so freely that tangential growth greatly exceeds radial growth, then the margin thickens and the limb, in radial section, will appear clavate from the centre outwards. It seems that this process occurs only in the long-lived fruit-bodies of *Fomes*, in which marginal growth proceeds in short bursts to give a series of concentric, raised zones on the surface of the pileus. In *P. xanthopus*, with the limb tapering much only near the margin, radial and tangential growth must be nicely balanced for the greater part of development, even while the direction of growth is approaching more and more to the horizontal.

It must not be overlooked that the shape of the limb depends also on the binding system, the radial growth of which causes the limb to thicken behind the margin. The co-ordination of marginal growth and the formation of the limb are in fact extraordinarily complicated by the presence of the different systems. The radial growth of the framework is due mainly to the skeletal system; tangential growth is due to the mediate and generative systems; and superimposed on the growth of the framework is the radial and tangential expansion of the binding system. Were only one kind of hypha present, the fact that radial and tangential growth would be performed by the same hypha would supply a natural regulation for the two processes; when a branch arose, apical growth in that hypha would then be retarded, and on the average every hypha at the margin would branch at the same rate, provided that they were equally supplied with food. When four systems are involved, the regulation of marginal growth means the co-ordination of the individual demands of each system on the one food-supply. The binding hyphae and the branching portions of the mediate and generative hyphae, being somewhat nearer the substratum, will be able to draw on the food-supply before the apices of the skeletal hyphae at the extreme margin, and this arrangement may tend to keep in check

the mechanism for radial growth. But the problem is difficult to evaluate without knowing what is the system of food-supply in the fruit-body; whether, for instance, the hyphae are so knit that they form a common sink from which each and every apex may draw its ration wherever in contact with another hypha, i.e. to what extent dissolved substances may pass from hypha to hypha across the walls; or whether the diffusion channels are confined within the lumina, in which case each family of hyphae derived as branches from one hyphal stock at the base of the stem will have its own isolated food-supply, and the generative hyphae which keep an open lumen will rank as most important in conduction. Considering the variety of hyphae which may compose a family, their varying combination in different families, and the different demands they will make on the food-supply according as they terminate in basidia, or as sterile ends, or in the marginal meristem, it is surprising that a regular and simple fruit-body can be the outcome. It is too easy to suppose that when the hyphae are numerous the irregularities will cancel out.

(e) In many fruit-bodies inequalities in marginal growth of the pileus give to the stem a position which appears excentric with respect to the circumference of the pileus. The inequalities are directly related to the inclination of the stem to the horizontal (cf. Text-fig. 15). If the primordial shaft has grown vertically from a more or less horizontal surface, it bears a centric pileus; if, at the other extreme, the shaft has grown almost horizontally, the stem is almost lateral and the pileus has only a rudimentary limb on the upper, inner side owing to the inhibition of marginal growth; and if the shaft has grown obliquely it bears a pileus more or less excentric, with a greater or less development of the limb on the inside towards the substratum. The degree of inhibition on the inside can be correlated with the angle which the direction of marginal growth makes with the horizontal, following upon expansion of the apex of the primordial shaft, and therefore ultimately with the direction of the stem. When the shaft lies nearly horizontal the margin of the pileus on the inside must begin to grow very steeply, vertically, or even more so, and on the outside it will grow nearly horizontally. When the shaft is vertical the margin grows out equally all round at an angle of about  $40^\circ$  with the horizontal. It appears that there is a *critical angle* of steepness for the direction of marginal growth. If this angle is exceeded, marginal growth cannot occur and, as it is approached, so the hyphae grow out less vigorously and the limb of the pileus is reduced and rudimentary. Thus the graded excentricity from inner margin to outer margin of the pileus borne on an oblique stem, and thus it follows that the margin will grow most rapidly in a horizontal plane. The hypothesis supports the contention that the limb is orientated by diageotropic sensitivity.

Buller has another explanation for the same phenomenon in *P. squa-*

*mosus*. Excentricity he correlates with geotropic curvature of the stem. The short conical primordium is negatively geotropic, and when it has grown obliquely a geotropic stimulus causes it to become vertical by increased growth on the under side. The apex then expands into the pileus, which develops excentrically by growing more rapidly on the outside, i.e. on the side on which growth has already been stimulated, than on the inside. When the primordium has grown vertically there is no geotropic curvature, and the pileus develops equally on all sides. In extreme cases when the short primordial shaft has curved through nearly 90°, no limb develops on the inside, and the stem is lateral. Hence marginal growth of the pileus is stimulated on the outside to an extent corresponding with that to which the same side of the stem is stimulated geotropically. In *P. xanthopus* I found no evidence of geotropic curvature of the stem, and the case of *P. squamosus* can be put as concisely, perhaps, by considering that the geotropic stimulus retards growth on the upper side of the stem and, in corresponding degree, inhibits growth of the pileus on the inside. The excentricity of the pileus in these two species can thus be accounted for on somewhat similar lines.

4. *The formation of the pores*. The peculiarities of pore-formation are these:—

(a) The pores are developed acropetally behind the growing margin of the pileus and normally on the under side.

(b) The pores are areas of inhibition, the dissepiments are paths of vigorous growth, in an evenly excrescent belt of narrow, interweaving hyphae, 1–2.5  $\mu$  wide.

(c) The pores are delimited at full width.

(d) Within narrow limits pores and dissepiments are of constant size; the pores are thus regularly placed and as crowded as possible.

(e) There is no secondary enlargement by vacuolation of the tissue-elements in the pore-field, which is characteristically a meristematic region.

How an external factor such as gravity or light can act formatively is hard to see. Pores do not develop on the upper side of the pileus in *P. xanthopus* because the binding and generative hyphae which give rise to the dissepiments do not penetrate so far; and where there is a field of the required character all round the primordial shaft behind the apex, pores develop uniformly on the whole surface. And in the case of *P. squamosus* Buller obtained normal fruit-bodies which had developed in total darkness, except for the short exposure to light of two days at a very early stage, which sufficed merely to initiate the pileus. Some internal mechanism must regulate the inception and arrangement of the pores.

5. *The orientation of the tubes*. The tubes are straight and point vertically downwards. They are oblique to the ascending portion of the

limb in the centre of the pileus, but they gradually become normal to it as it becomes horizontal towards the margin.

The spores, being borne on the inside of the tubes, are dispersed by air-currents after they have fallen a greater or lesser distance through the tubes, and unless these narrow passage-ways are orientated in line with the direction of gravity the advantages of an increased area of spore-production, gained from their formation, will be lost through interception of the spores, at least from the upper parts of the tubes, before they can reach the exterior. But there is ample evidence that the mechanism is well controlled, that the hyphae of the dissepiments are positively geotropic. It would suffice to observe the direction of the tubes in wild fruit-bodies, especially such as have grown from oblique surfaces: how they always point vertically downward and how in perennial fruit-bodies, when the support has fallen over or been displaced, the successive pore-layers have a different orientation which represents the vertical in the different positions. But Buller (4) has recorded the positive geotropism of the tubes in *Fomes fomentarius* and *Ganoderma applanatum* and Long and Harsch (8) observed that the axes of the tubes of various polyporoid primordia developed in artificial culture were always in line with the direction of gravity, though with the curious provision that the tubes, when formed on an upper surface, opened vertically upwards.

In *P. xanthopus* the dissepiments are so short that some obliquity of the tubes will not seriously affect the discharge of spores, but in species with long narrow tubes the power of adjustment must be exceedingly delicate. For fruit-bodies of *G. applanatum*, in which the tubes are 10–20 mm. long and 170  $\mu$  wide, Buller has calculated that with a deflection of 1° from the vertical the spores from the upper five-sixths of each tube will fall upon the side of the tube and fail to clear the opening.

The apices of the binding and generative hyphae which form the dissepiments must be the parts which respond to the geotropic stimuli, because there is no means of altering the direction of the hyphae or the set of organs once they have been formed. Now the apices of the skeletal and generative hyphae at the margin of the pileus are sensitive in some degree to gravity, for they grow diageotropically, but this cannot be the case with the binding and interweaving generative hyphae which grow indiscriminately whether in the tissue or from the under surface immediately behind the margin. One preliminary, therefore, to the formation of the tubes must be the sensitizing of the tips of the binding and generative hyphae in those parts of the pore-field in which dissepiments are to develop. Perhaps also the hyphal tips over the pore-areas are similarly affected, but I have seen no clear indication of alinement of the hyphae in these places; it would explain how the basidia derived from the apical cells of the generative hyphae at the top of the tubes come to point down-

ward, but this property can be explained in another way, as will be shown presently. One must conclude that there is a force in the pore-field which is confined to the paths of the future dissepiments; and a diageotropic generative hypha in the pileus may bear a lateral which, after an interval, without response to gravity, may approach within the influence of this force and become positively geotropic.

6. *The cessation of downgrowth of the dissepiments.* The tubes are very short in *P. xanthopus* because the downgrowth of the dissepiments soon comes to an end. A common pore-level, at a certain distance from the flesh, is attained about 3 mm. behind the margin of the pileus, and such a level is a feature of all polyporoid fruit-bodies in which the dissepiments are regular and of limited growth. The distance behind the margin at which it is attained depends chiefly on the duration of downgrowth, or the length of the dissepiment; this is a property which should prove of systematic value. In some species, like *P. xanthopus*, the length of the dissepiment is remarkably constant, yet in others it varies considerably from fruit-body to fruit-body and in many in different parts of the same fruit-body. If the property were merely the outcome of the nutritional arrangements in the fruit-body, i.e. that the hyphae in the dissepiments stopped growing because they could no longer obtain sufficient food, then the dissepiments should always be longest in the oldest, basal or central, part of the pileus, which is nearest the food-supply, and they should gradually decrease in length towards the margin. Such a configuration is unusual. The dissepiments generally stop growing at a point closer to the food-supply than the margin of the pileus and the pore-field, which continue in activity. Nor can an external factor be effective. Dryness of the air might stunt the dissepiments, but given a moist atmosphere and other conditions requisite to the growth of the fruit-body and the tubes will have their characteristic length. Again an internal factor must control the length of the dissepiment.

Behind the growing margin it seems there must be generated lines of force which, advancing through the pore-field, lay down the paths of continued outgrowth. And, as they proceed, these lines of force must continually change direction; they fork and join with neighbouring lines to advance and fork again; and thus they plan the network of dissepiments while the intervening areas persist unstimulated as the pores. It seems that an impulse travels radially, that it is confined in narrow, changing channels, and that it stimulates to further outgrowth the binding and generative hyphae which lie along its course and leaves them positively geotropic. The factor which controls the length of the dissepiment will be connected closely with this mechanism: perhaps the length is determined by the intensity of the force which generates the dissepiment and in such a manner that a high intensity provokes a long-continued outgrowth

and makes a long tube, while a low intensity would make a short tube. Or there may be a peculiar arrangement whereby a general outgrowth in the pore-field is checked in certain places which are equidistant and of constant size, and then the dissepiments will just appear to circumvent the pores. But I think not, for it is apparent that the outgrowth of hyphae in the dissepiments is not of the same character as that in the pore-field, and it would be difficult to explain the angular outline of the pores on delimitation by means of this hypothesis. However, there can be no doubt that a study of the dimensions of the dissepiments, of the shape of the very young pores, of the distance from the margin of the zone of inception of the pores and of the part where the dissepiments stop growing, in any of the common polymorphic species, *Lenzites saepiaria*, *Daedalea flavida*, or *Irpex*, for example, or in one with large pores such as *Hexagonia*, would throw much light on the nature of the forces playing in the pore-field.

7. *The constriction of the pores.* The hyphal tips, before coming to a standstill at the end of their downgrowth as the dissepiments, turn aside, interweave a little, and partially occlude the pores. As they escape from the force which generated the dissepiment, so it seems that they lose the power which it conferred upon them and they revert to an insensitive or diageotropic condition.

8. *The formation of the disc.* The fruit-body is attached to the wood by the narrow strand of hyphae which joins the central core of the stem with the mycelium. To prevent it from falling over there must be an additional support at the base. This is probably the function of the disc, to act as a buttress; for when the stem is vertical the disc is equally developed round the base, but when it is oblique or horizontal the disc is thickened on the lower side. The outgrowth of the binding hyphae beyond the general surface of the shaft may be a response to the strains transmitted to the base of the stem, as the fruit-body grows farther and farther from the substratum; such behaviour agrees with the argument that this system serves primarily to consolidate the skeletal framework. But the hyphae may respond also in part to contact with the wood. One can often detect a thigmotropic response where a hyphal tissue, rhizomorph, or a developing fruit-body, has encountered a hard body, for it often grows on firmly adherent to the surface.

Certain of the more conspicuous anatomical factors may also briefly be dealt with:

1. The development of the pigmented crust on the stem and pileus may be due to the oxidation and illumination of the surface. It seems also that the tissue degenerates through senescence, because the formation of the crust involves more or less complete disorganization of the hyphae, which are probably dead at maturity of the fruit-body.



2. Commonly the hyphae grow out from the base of the fruit-body in basidiomycetes and form a secondary mycelium or a villosity round the point of attachment. Although a few behave in this manner in *P. xanthopus*, most are stimulated to a slight degree only to form a palisade, and in the process they assume the properties of skeletal hyphae in the same way as the binding hyphae which form the dissepiments: it is noteworthy that in each case they are roughly of the same length. The secondary mycelium in this fungus is rudimentary.

Superficial outgrowths of this kind are frequently caused by excessive humidity and stagnant air, but the appearance of the velvet in *P. xanthopus* was the same, whether the fruit-bodies were grown in a very damp atmosphere under a bell-jar or under drier conditions in the open room. The formation of the velvet must be referred to an internal factor which is curiously restricted in action to the base of the stem.

3. The consolidation of the skeletal system as a cylinder in the stem and as a fan-plate above the tubes in the pileus may be a matter of oxidation, or it may represent the activity of the binding system in a region of optimum food-supply.

4. The development of the hymenium over the surface of the tubes may also be a matter of oxidation. The apices of the generative hyphae may be attracted by high oxygen concentration into the space of the tubes and, as soon as a high enough tension is realized in the apical cell, it enlarges into a basidium. The basidia tend to point towards the opening of the tube, i.e. inward to the centre and downward, and this direction corresponds with the route of increasing oxygen concentration in the air of the tubes, or, inversely, of decreasing concentration of carbon-dioxide.

Yet further, under the heading of histological factors, might be discussed the interrelations of the different hyphae; why, for instance, one kind of hypha, or a lateral from it, is transformed into another kind by losing certain properties and acquiring others, and why there is not an infinite variety among the hyphae; or how one and the same hypha may vary in geotropic, phototropic, or other response at different periods in development of the fruit-body. But these problems are too abstruse and are concerned, no doubt, with cytoplasmic factors. Only two points must be mentioned.

A branch from a binding hypha or a mediate hypha often arises where the wall is thickened, and the extrusion of the lateral thus indicates a certain fluidity of the matter composing the wall. And secondly, there appears to be a vestigial apical cell mechanism in certain of the skeletal hyphae of the pileus. The mechanism differs from that in the generative hyphae in that the septa are unaccompanied by clamps, and they are quickly reabsorbed. It would be interesting to know if nuclear division precedes

separation. All trace of the mechanism has disappeared from the tapering skeletal hyphae in the primordial shaft.

*The Biology of the Fructification.*

*Rate of growth.* I made several observations on the rate at which the fruit-bodies developed from pieces of wood under bell-jars; they refer mostly to the primordial shaft.

*The Rate of Growth of the Primordial Shaft.*

Initial Length. mm.	Final Length. mm.	No. of Days.	Average rate of Growth. mm. per diem.
1.5	37.0	19	1.9
A. 2.0	34.0	22	1.5
0.5	22.0	16	1.3
B. 1.0	15.0	14	1.0
1.2	15.0	11	1.3
C. 2.0	13.5	7	1.6
D. 2.0	12.5	12	0.9
0.3	12.0	9	1.3
0.2	11.5	11	1.0
0.5	10.5	8	1.3

In all cases the shaft grew roughly at the same rate, at an average of 1.3 mm. *per diem* of 24 hours. The observations, made at intervals of 24 hours, and in a few cases of 12 hours, gave some indication that the rate varied, though not at any definite stage in development. Thus in the case of A, in the accompanying table, the shaft grew at a rate of 2.5 mm. *per diem* from a length of 7 mm. to 18 mm.; in the case of D, the shaft grew from 2–8 mm. at the rate of 2 mm. *per diem*; in the case of B, however, growth continued at 1 mm. *per diem* for the whole period of observation, and that of C continued steadily about 1.6 mm. *per diem*.

The rate of growth of the pileus was measured daily in four cases, and each gave a daily increase of about 1.0 mm. in radius, i.e. in the distance between the stem-apex and the margin. The pilei were all rather small, and after reaching 9–15 mm. in radius they stopped growing.

The primordial shaft and the pileus grow as well in the daytime as during the night.

*Longevity.* I have no direct observations on this point. The following records were taken from fruit-bodies grown under bell-jars, and cover the period of active growth from primordia 0.5 mm. high; the measurements refer to the final size of the fruit-body.

Size of Stem. mm.	Radius of Pileus. mm.	No. of Days.
12 × 2.5	11.5	18
13 × 1.5	9.0	24
22 × 3.0	15.00	31

From the average growth-rates obtained for the primordial shaft and pileus one would put the age of a well-grown fruit-body, of stem 25 mm. and pileus 40 mm. radius, at 59 days, or about 2 months: i.e. 19 days for the stem and 40 days for the pileus. This seems a very long time, and with optimum conditions in the jungle growth may be quicker: the pileus of a very large specimen in the Singapore Herbarium is 19.5 cm. in diameter, and it would have taken 3 months to develop at the rate of 1 mm. *per diem*.

*Sporing period.* Sporing may begin a few days before the pileus develops from basidia in the cauline pores near the apex of the primordial shaft, but in most cases not until the pores have developed on the pileus. Sporing continues night and day so long as the pileus is growing, which may be a matter of 1–2 months. The fruit-bodies continue to spore for some time after they have stopped growing and after they have lost connexion with the mycelium, by drawing upon their own reserves. Thus 9 medium-sized fruit-bodies, gathered freshly from the jungle at different dates, were detached from the wood and put to spore in a damp atmosphere, being occasionally moistened with water. Of these nine, two spored for 10 days, one spored for 35 days, and the rest for varying periods of 16–25 days. Two to five weeks can, therefore, be taken as the reserve sporing-period of the mature fruit-body.

In the jungle I had noticed that the largest fruit-bodies were to be found in places which were always damp, such as by watercourses in the deep shade. Those developed in secondary jungle, or places which were apt to dry out at frequent short intervals and which were continuously humid only for a month or so at a time, were always rather small. It would be interesting to know how the fruit-bodies survived desiccation. Accordingly I collected thirty odd specimens, dried them in the air at room-temperature which varied from 75–85° F., and after varying intervals soaked them out and put them to spore. Four of the fruit-bodies dried for 90 days, four for 60 days, two for 52 days, two for 42 days, and three for 38 days, failed to revive but became mouldy. On the other hand, five fruit-bodies dried for 12 days, two for 14 days, two for 18 days, and four for 21 days, spored heavily on soaking out. Two fruit-bodies dried for 28 days spored less abundantly, and three dried for 35 days scarcely spored at all. One may conclude that the fruit-bodies cannot survive desiccation for more than five weeks, and even after three weeks they are somewhat enfeebled. For periods up to three weeks, however, they retain full vitality: in many cases they began to spore only 12 hours after soaking out.

Some other fruit-bodies I dried for two and three consecutive periods of two weeks with a short interval of one to two days' sporing, and not until the third drying, and in two cases the fourth, did they fail to revive. In these

experiments after the second drying, as well as in fruit-bodies dried for more than twenty days, I noticed that sporing was usually confined to the region near the margin of the pileus, as if the older tubes had suffered first and had become effete.

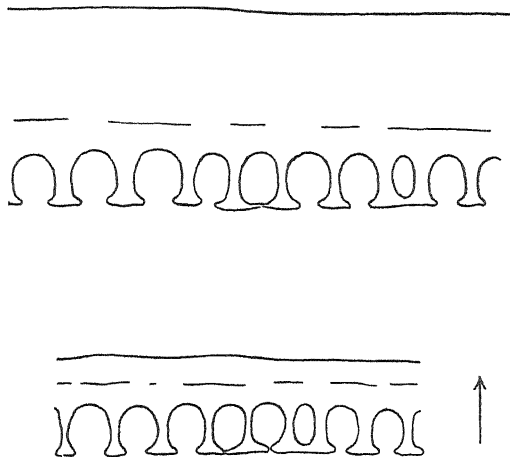
The margin of the pileus never grew afresh in fruit-bodies which had revived after an interval of desiccation, whether or not they were attached to the substratum. Nor have I seen any sign of discontinuous growth in wild specimens. Once the margin has been dried up, it cannot grow again; and this inability confirms the observation that large specimens are always found where growth can proceed uninterrupted.

The most remarkable fact about the behaviour of this fungus relates, however, to detached fruit-bodies which have been sporing for some time. They grow thinner and thinner, till eventually when sporing stops they have become pliant and papery with soft spongy stems and they dry up as brittle as parchment. Nearly all the tissue which lies between the tubes and the pellicle of the pileus disappears, and the pellicle can be stripped off in sheets from the tubes on to which it has gradually sunk; it becomes wholly detached save at the margin and the centre. The gross effect of starvation can be seen in Text-fig. 16. On microscopic examination the thick walls of the skeletal and binding hyphae are found to have been eroded away excepting in the pellicle, the dissepiments, and a thin layer immediately above them; even those in the stem suffer to a greater or lesser extent. The matter in the walls is eroded from the *outside* inward to the lumen, sometimes irregularly, sometimes fairly evenly, and finally it all disappears, leaving the hypha like a narrow structureless thread or wisp of cellulose, 1–2  $\mu$  wide, and no bigger than the original lumen. One can see in the process that the eroded matter lies between two membranes or sheaths, one on the outside and the other next the lumen, somewhat like the myelin of a medullated nerve-fibre (Text-fig. 17). As the matter disappears the outer sheath collapses on the inner one, and then the inner one collapses on the lumen. This is not the usual picture of the structure of a hyphal wall that one imagines, nor would one expect the cell-contents to remove the deposit in the wall by beginning on the outside.

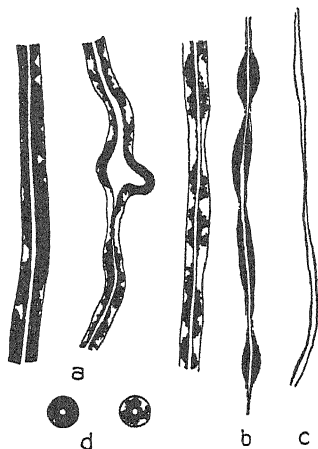
One cannot but infer that when it was detached and sporing the fruit-body drew upon reserves which had been deposited during development in solid form between the two sheaths of the wall, and that these reserves are translocated *via* the dissepiments to the basidia and thence into the spores. The distant reserves in the stem are also mobilized. Now, unfortunately, all my specimens grew mouldy when they had become very thin, yet, could they have been kept free from mould, there remains the strange possibility that they would have vanished in a cloud of spores.

Old specimens in the jungle are often limp and papery, as if they had consumed a large portion of their own substance.

*Xerophytic mechanism.* Compared with many polypores, the fruit-bodies of which can withstand desiccation for months or years, those of *P. xanthopus* are not particular, but they show quite clearly the xerophytic



TEXT-FIG. 16.



TEXT-FIG. 17.

TEXT-FIG. 16. Sections from corresponding parts of a normal pileus (upper figure) and of a pileus which has been starved, though actively sporing, for three weeks (lower figure).  $\times 50$ .

TEXT-FIG. 17. Fragments of hyphae from a starved fruit-body: *a*, successive stages in the irregular erosion of the wall; *b*, a fragment in which erosion has proceeded evenly in certain places; *c*, a fragment in which the wall has disappeared except for the two sheaths; *d*, the cross-section of a normal and a partly eroded hypha.  $\times 1,000$ .

mechanism which has been adopted generally by this class of fungus. The crust on the stem and the pellicle of the pileus seem fairly impervious to water, and they will afford a certain amount of protection against evaporation. The fruit-body thus dries out mainly through the pores, and the closing of the mouths of the tubes, which in *P. xanthopus* are exceptionally short and the more likely, therefore, to lose water, may suffice to protect the hymenium over a hot day or two. The chief xerophytic factor, however, lies evidently in the narrow lumen and thick, rigid walls of the hyphae (Text-fig. 17 *d*). Consider the effect of drying such a hypha. Water evaporates from the walls, and the walls must draw water from the cell-contents. The cell-contents must shrink, in which case either the walls must collapse or air must be sucked into the cell-cavity, or a vacuum will appear. The cohesive power of water confined in a narrow closed tube will prevent the formation of a vacuum; the walls of the hypha are too thick and rigid to cave in; and the force necessary to suck air through the damp walls is certainly much greater than that which withdraws the water. The force of evaporation will depend on the difference between the pressure of water-vapour in the air and in the cell-cavity, and certainly it is not large enough to overcome any of the three resistances opposed to the retraction of

water. Thus, beyond a slight tension due to incipient drying of the walls, the cell-contents are enabled to retain almost their full store of water. Air-bubbles are never seen inside the thick-walled hyphae of woody polypores, and why the fleshy fruit-bodies of such as *Polyphorus sulphureus*, *P. fragilis*, or *P. Schweinitzii* cannot survive desiccation is probably because the hyphal walls are thin and collapse on to the cell-contents, which, therefore, continue to lose water until the safety limit has been passed and the cytoplasm disorganizes. Nevertheless, a problem remains how the generative hyphae survive desiccation to produce new basidia on the advent of water.

*Spore germination.* The spores germinate readily in a film of water, and in 24 hours they will have produced from one end a narrow aseptate germ-tube,  $1-1.5\ \mu$  wide, and up to  $30\ \mu$  long. I have not inquired into their ability to resist desiccation.

#### CONCLUSIONS.

In basidiomycetes which have the hymenium so elaborated that the spores are discharged between closely packed gills, serried spines, or through narrow tubes, it is most important for the accomplishment of this feat that the fruit-body should possess a high degree of rigidity in addition to a power of directional growth and precise orientation of the hymenial surfaces. Buller has proved and exemplified the need beyond a doubt (3, 4). In the fleshy fruit-bodies of agarics, of *Boletus* and of *Hydnum* this end is attained by the turgidity of the tissues and the tensions which are thus developed, but the polypores, it would appear, rely mainly, if not entirely, on the thickened walls of the hyphae and the formation of 'crusts': apart from the sappy, fleshy kinds, there are probably no tissue-tensions arising from turgidity in polyporoid fruit-bodies. Now in this case, with growth well directed and an exact orientation, such as becomes an efficient fruit-body, the hyphae will be longitudinal and but slightly interwoven, like a bundle of twigs, and there will be very little cohesion in the tissue. A fruit-body of this structure could not reach a size of more than a few millimetres without additional support for its members, because it would fall to pieces under its own weight. Such is the nature of the skeletal framework in *P. xanthopus*, and the additional support is clearly derived from the binding system and the agglutination of the superficial hyphae into a crust over the stem and a kind of pellicle on the upper side of the pileus. As a result the hymenium can be elevated well above the substratum and securely depended from a rigid structure which is also strong enough to escape being damaged or upset by the heavy raindrops, the falling twigs and leaves, or by an animal brushing past. How weak the fruit-body and prone it is to collapse without these mechanical factors can easily be imagined: I doubt if a skeletal hypha several centimetres long could support itself

in a vertical position, and far less when the upper half is bent over to the horizontal, though, of course, in a tissue it derives some mutual support from the slight entanglement. It is interesting, too, to discover the part which the unseptate hypha can play in a eumycete.

One may ask, nevertheless, if the required strength could not have been obtained as surely by means of the ordinary, branching, septate hyphae, with slightly thickened walls. A more complicated system of hyphae can hardly be expected in a polyporoid fruit-body, but one cannot suppose that this fungus is very exceptional. There are several other tropical species in the section Microporus which are closely related. They differ superficially in having a spatulate or flabelliform pileus with a lateral stem, a deeper coloration, and a greater though varying degree of pubescence. Microscopically they may agree in structure, or they may be less specialized, and perhaps they will reveal how the multiple system of hyphae has arisen from the unitary. It will be interesting to know also how the simple mesopodal forms in other sections compare in structure, for example, *Polyporus perennis* in Pelloporus, *P. nummularius* in Melanopus, or even *P. rugosus* in Amaurodermus.

If the fruit-body of *P. xanthopus* is so specialized in microscopical construction, it may be specialized in other respects. The very thin flesh and the short minute tubes, that is the general slightness of the fruit-body, might be considered as a primitive mark, yet with equal reason as a reduction from a massive form. It must be clear that without knowing the forms which are taken by fruit-bodies based on a single hyphal system one cannot draw a satisfactory conclusion about the evolution of the polyporoid fruit-body.

As for the development, Lloyd was mistaken when he wrote of this fungus that 'the pileus is formed at a very early stage, and both the pileus and stem grow concurrent' (7). When the apex of the primordial shaft dilates to form the pileus, the rest of the shaft becomes the stem and, there being no secondary means of elongation by vacuolation or interstitial growth, the inception of the pileus must determine the length of the stem. Lloyd figures the specimens on which his conclusion was based, and they are evidently young fruit-bodies with precocious development of the pileus: thus the stem in both is rather short, and compared with ordinary specimens it does seem but half-grown.

The supposed influence of external factors on the development of the fruit-body may conveniently be summarized by postulating the form which the fruit-body should assume under certain specified conditions. Thus, when developed in darkness, the fruit-body should be cylindrical, straight, unbranched, apileate, colourless, crustless, and covered with pores, and perhaps fertile. Or, when grown on a klinostat to eliminate the effect of gravity, it should depart from the normal only in that the pileus should be

regular and funnel-shaped whatever the direction of the stem, and the dissepiments should be abortive and probably sterile. These experiments I hope it will be possible to perform.

#### SUMMARY.

*Polystictus xanthopus*, Fr. lives in the tropics of the old world as a saprophyte on fallen twigs and branches. In Malaya it is common from sea-level to mountain-tops at 6,000 ft.

A specific description based on living material is given, and the structure of the fruit-body is described in detail.

Four systems of hyphae are employed in the construction of the fruit-body, namely: unbranched, thick-walled, aseptate, longitudinal, *skeletal* hyphae; branched, thin-walled, septate, clamped, *generative* hyphae; branched, thick-walled, aseptate, interweaving, *binding* hyphae; sparingly branched, aseptate, thick-walled, longitudinal, *mediate* hyphae.

The skeletal hyphae build a framework which the binding hyphae consolidate into a rigid cylinder in the stem, and a plate-like layer immediately above the tubes in the pileus. The generative hyphae give rise to skeletal hyphae through the mediate hyphae, to some extent also to binding hyphae, and to the hymenium. The hyphae of the dissepiments are derived from the binding and generative systems, and they assume the character of skeletal elements. The superficial hyphae are agglutinated into a crust or pellicle over the stem and on the upper side of the pileus.

Development is direct. A cylindric primordial shaft grows out to form the stem, and then dilates apically into the pileus. There is no motor-tissue or secondary means of elongation; the position of the stem, of the pileus, and of the tubes, the set of the whole fruit-body, is defined once and for all by the direction of growth of the apices of the constructive hyphae.

The primordial shaft is positively phototropic, but insensitive to gravity. The margin of the pileus is probably diageotropic. The hyphae which form the dissepiments are positively geotropic.

The form-factors of the fruit-body are examined in detail. The shape of the pileus is referred to two processes, one causing *expansion* or multiplication of the hyphae at the apex of the primordial shaft, and the other causing the formation of a *dead space* or area at the apex of the shaft over which further outgrowth of the hyphae is inhibited. The inception of the pileus is referred to a photic stimulus, and the orientation and eccentricity to geotropism. But the tubes must be generated by an internal mechanism operating in a region termed the *pore-field*, which is situated immediately behind the growing margin of the pileus on the under side and which travels radially *pari passu* with marginal growth. The nature of this mechanism is discussed. The factor controlling the downgrowth of the dissepiments



is likewise considered to be internal and closely connected with this mechanism.

The primordial shaft grows at an average rate of 1–1.5 mm. *per diem*, and the margin of the pileus at a rate of 1 mm. *per diem*. These data give a period of two months for the development of a medium-sized fruit-body.

Spores are shed while the pileus is developing, and probably for some time after growth has stopped. Sporing may also continue for two to five weeks after removal of the fruit-body from the substratum when the fruit-body draws upon its own reserves; it consumes the substance which has been deposited in the walls of the skeletal and binding hyphae.

Fruit-bodies dried for a period up to five weeks will revive and spore on moistening with water, but after three weeks of desiccation their vitality is appreciably reduced. Marginal growth is not resumed on revival of the fruit-body after desiccation.

The chief xerophytic factor is considered to be the thickening of the hyphal walls. The rigidity thus obtained, and the high impermeability of the walls to air, will prevent in large measure the detraction of water from the narrow lumen.

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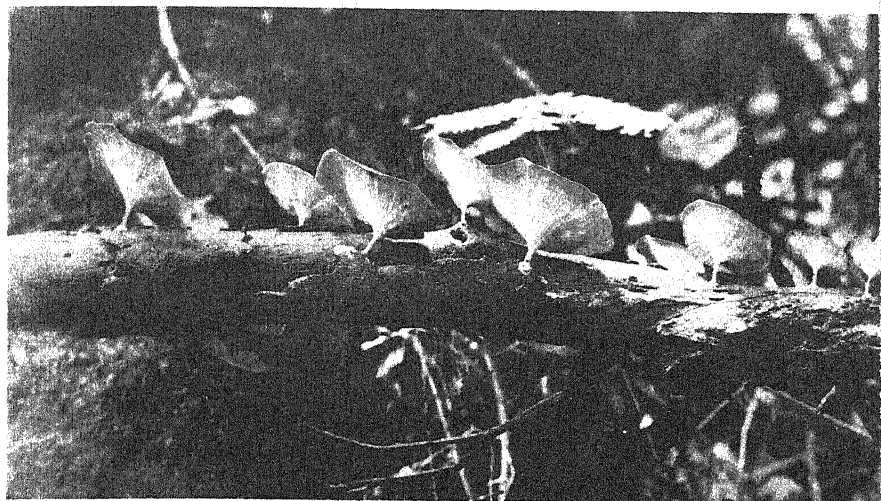
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#### EXPLANATION OF PLATE V.

Illustrating Mr. E. J. H. Corner's paper on the Fruit-body of *Polystictus xanthopus*, Fr.

Figs 1 and 2. Photographs taken in the jungle to show the habit and growth of the fruit-bodies of *P. xanthopus*.





1





# Observations on the Development of *Taonia atomaria*,<sup>1</sup> Ag.

BY

WILFRID ROBINSON, D.Sc., F.L.S.

With Plate VI.

*TAONIA ATOMARIA*, a member of the Dictyotaceae, is of somewhat rare occurrence on the British coasts, but, both here and elsewhere, the plants found have usually borne tetrasporangia. Lloyd Williams (8) has shown that a reduction division occurs in the development of the tetrasporangium. It has therefore generally been assumed that the alternation of generations established for Dictyota also holds for Taonia. Sexual plants have, however, very rarely been seen, the only record for Britain being that of Lloyd Williams (7) who described the motility of the antherozoids from plants collected at Llandudno. The same author, in a later publication (8), mentions that he has not observed sexual plants subsequent to that occasion. Sauvageau (6) comments on the rarity of sexual plants, but describes the antheridia from material collected by him in August of 1895 and 1896 at Guéthany, and in September 1895 at Cap Torres near Gigon (Spain); oogonial plants were apparently not found by Sauvageau. Reinke (5), who studied Taonia at Naples (1878), was unable to find the male plants, although one of his figures indicates structures which he suggests may be oogonial and antheridial sori.

More recently Funk (2) has stated that although the plant occurs abundantly at Naples, he has never found sexual plants. But shortly after the commencement of the present work, Von Ubisch (9) described strongly developed male plants of Taonia which she received from Naples in 1927. Her description of the antheridial development confirms the earlier account by Sauvageau. Figures are also given by Von Ubisch of supposed oogonia,

<sup>1</sup> This paper was written in the main by the late Professor Wilfrid Robinson. The parts in square brackets have been added by myself in collaboration with Miss Rachel Harries, M.Sc., who carried out the greater part of the laboratory work under Professor Robinson's direction. We have also written the summary, compiled the bibliography, and inserted the references to the plate. For this part of the work we take full responsibility, although we have endeavoured to express the substance of Professor Robinson's views as expressed to us from time to time during the course of the investigation.—LILY NEWTON, Botanical Department, Aberystwyth.

but from the figures these appear to me almost indistinguishable from the tetrasporangia, although it is stated that there are differences in shape and arrangement. Since the germination of fertilized eggs was not observed by Von Ubisch, the matter seems to require confirmation.

From a consideration of the published literature, it is thus obvious that there is a considerable difficulty in accounting for the predominance in nature of asexual plants of *Taonia*, and for the paucity of the asexual plants even in localities in the warmer seas where *Taonia* is abundant. It may be recalled, however, that there is a somewhat similar condition in regard to the relative abundance of the asexual plants of *Dictyota* (8) in some localities, and parallel observations have been made on the occurrence of *Padina pavonia* in Britain (1). The facts would appear to suggest that in certain localities for *Dictyota* and *Padina*, and in most localities for *Taonia*, the conditions are such that the full development of the sexual plants arising from tetraspores is inhibited. The perpetuation of the asexual plants bearing tetraspores must, therefore, be brought about by some special means. Such a means of vegetative propagation is provided by the perennation and growth of the rhizome-like branches proceeding from the lower region of the thallus in *Padina* and *Dictyota*. In *Taonia*, however, such structures have not been described by previous investigators, nor has the present work revealed the existence of vegetative branches capable of propagative function. The means of perpetuation of the asexual plants of *Taonia* must, therefore, be sought elsewhere.

[Later work has shown the occurrence of plantlets on the basal rhizoids of material, *in situ*, in Cardigan Bay.]

The material used in the present work occurs in rock pools at the mid-tide level at Aberystwyth. The plants first found in September 1928 were large, fully-grown tetraspore-bearing plants, which exhibited all the features described for mature plants by Reinke. They were attached to the sides of the pool by a densely matted filamentous growth of branching rhizoids. There is no solid rhizome-like base as in *Dictyota*, yet the matted rhizoids, incorporated with fine particles of mineral matter, give the appearance of a cushion-like base. The thallus in the specimens found was flattened and much branched, and was crossed by transverse bands giving a zoned appearance. The zonate bands are due in part to lines of hairs, but also to the production of tetrasporangia in greater abundance in the vicinity of these lines of hairs than elsewhere. Contrary to the statement of Von Ubisch (9) regarding the published figures of Reinke and of Rabenhorst, Pl. VI, Fig. 1, of a tetrasporic plant, shows the figures of the former workers substantially correct for most plants. It may be stated, however, that since tetrasporangia are not confined to the bands, but are usually also to some extent scattered in lesser numbers between the denser zones, examples may be found in which the zoning is less obvious than in others. This becomes

especially evident towards the end of the season in the vicinity of the apical region of the thallus (Pl. VI, Fig. 2).

The growth of the thallus takes place by an apically placed marginal meristem (Pl. VI, Fig. 17), which gives rise to the cells of the limiting and medullary regions by regular divisions. These divisions must take place simultaneously along the apex, giving rise to rows of cells which, for the most part, retain their regular seriation. The appearances suggest a rhythmic production of the bands of hairs and tetrasporangia by pre-determination at the apex (Pl. VI, Fig. 1), but clear evidence was also obtained for the subsequent development of tetrasporangia, between the more definite zones, from cells which were not predestined at the apex to be tetrasporangial (Pl. VI, Fig. 3). The two different modes of origin of tetrasporangia were well seen in a piece of the apical region of the thallus cultivated in sea-water in the laboratory. In this case the development of a band of hairs and tetrasporangia was traced from the first signs of fertility, which was manifested in about the twelfth row of cells behind the apex and exactly parallel to it. Under the cultural conditions, which were probably more uniform as regards illumination than the sea, it was found that only one or two such bands appeared. Subsequently, after several weeks (six or eight), tetrasporangial rudiments were developed from any of the outer cells of the thallus, and indeed ultimately at the margin and from the apex itself.

The rhythmic production in nature of tetrasporangia in bands strongly suggests phases of purely vegetative growth alternating with phases in which the reproductive cells are initiated. An explanation for such alternation may be found in the necessity for a period of vegetative activity, leading up to the accumulation of material necessary for the production, by the meristematic cells, of tetrasporangial rudiments. Such accumulated material may then be largely utilized in the initiation by the apex of the reproductive cells, when a further period of vegetative activity leads to the separation of the initiated reproductive cells in a zone. Further observations during the summer of 1929 have proved that the development of the *Taonia* plant from microscopic size to a fully mature thallus of about 8 inches takes place in about thirty days. During this period some sixty zones of tetrasporangia may be formed. It would thus appear probable that there is a definite correlation between the daily tidal rhythm and the initiations of the zones of tetrasporangia. Since there are also striking rhythmic differences in the density of the tetrasporangia in adjoining zones it may be provisionally suggested that these differences are correlated with the different amounts of light received by the apical margins in the successive inter-tidal periods. It is obvious that given fine weather the period of effective illumination will be much greater at the spring tides than at the neap tides. But it must also be stated that the development of tetra-

sporangia from cells between the more definite zones, together with the development of tetrasporangia from any cell of the thallus under cultural conditions, suggests that the zonation may arise from a greater susceptibility of apical cells to the periodic illumination than the more mature cells of the thallus.

Periodicity in the production and maturation of the antheridial sori of *Dictyota* has been observed and studied by Lloyd Williams (7), Hoyt (3), and Lewis (4). This periodicity has to some extent been related to tidal periodicity during each lunar month, and Lloyd Williams (7) has suggested that the incidence of illumination during the preparatory periods may be a casual agent in the periodicity. No periodicity of the production of tetrasporangia appears to occur in *Dictyota*. It would seem that the zoned distribution of the tetrasporangia in *Taonia*, and possibly also the periodicity in the antheridial sori of *Dictyota*, may be explained on the bases of rhythms dependent on the accumulative substance, before reproductive structures can be laid down behind the apex, and the withdrawal of such substance, necessitating a further period of vegetative growth before the next zone (or crop) of reproductive structures is laid down. That tetrasporangia subsequently arise from cells between the zones can be understood if the necessary substances are products of photosynthesis, since it is obvious that such cells may also gradually accumulate sufficient material to allow of their becoming tetrasporangia. [This paragraph bears a question-mark in the manuscript.]

The above explanation becomes more feasible when considered in relation to the known stimulation of vegetative growth by increasing the proportion of nitrogenous nutrient, or, in other words, by diminishing the *C/N* ratio, as undoubtedly will follow, on the large accumulation of carbohydrate material in the tetrasporangia of *Taonia*.

On this view any external factor tending to disturb these relations would modify the rhythm. Increased illumination as at the time of spring tides or increase in the nitrogen supply in the water would operate in this way. It is noteworthy, in the light of such considerations, that the intervals between successive zones of tetrasporangia show striking inequalities, and since the numbers of zones on the plants collected in September suggested a possible daily periodicity, the inequalities may be accounted for by the different periods of illumination dependent on the tidal fluctuations. It is hoped that direct observations on marked plants in the sea during the growing and reproductive seasons, together with cultural experiments, will lead to an elucidation of this matter. It has, however, been mentioned above that, in preliminary experiments, in which pieces of thallus, including the growing apex, were grown in the laboratory with illumination (which was, of course, more uniform from day to day than in the sea), in sea water to which nitrate and phosphate were added at intervals, the rhythm and



production of tetrasporangia came to an end. It was, however, replaced by the development of tetrasporangia by most of the outer cells of the younger and apical portions of the thallus.

*Description of the Tetrasporangia as found.*

Collection of mature thalli of *Taonia* at Aberystwyth usually showed the tetrasporangia in the undivided condition, but on certain days at the time of the spring tides in August and September a proportion of the tetrasporangia showed tetrads. Pl. VI, Fig. 4 shows the first division of tetrasporangia. In other cases the tetrasporangia had liberated their contents (Pl. VI, Fig. 5). Adhering to the surface of such plants there was usually an abundance of germlings in all stages of development, from two or three cells up to plantlets of 5 to 20 mm. in length. The younger germlings adhering to the surfaces of the mature plants gathered in September 1928 were of two distinct types. This observation suggested that they might be of different origin. Laboratory cultures were therefore begun in order to trace the development of the germlings. Whole plants were kept in glass dishes in filtered sea-water, and from these plants small clean portions bearing tetrasporangia were transferred into filtered sea-water in shallow glass Petri-dishes. The cultures were kept in moderate light on the laboratory bench. Under these conditions, in the course of two or three days, the contents of the tetrasporangia began to be liberated.

It was at once noticed that bodies of two different sizes were being liberated from the tetrasporangia; there were obvious tetraspores, which often remained loosely aggregated in groups of four, but in addition there were larger bodies, which approximated in size to the whole undivided contents of the tetrasporangium. Thus it became clear that while some of the tetrasporangia had divided into four tetraspores, presumably following a reduction division, many others liberated the unsegmented contents as a single spherical cell.

The germination and subsequent development of these two types of cell from the tetrasporangium has been followed in culture for several months.

The tetraspore first becomes segmented by a transverse wall into two cells of equal size. One of the cells immediately grows out to form a rhizoid-like filament (Pl. VI, Fig. 6), which soon elongates with further divisions. The cell of this first rhizoid filament, as well as those which originate later, are distinguished from the cells at the opposite pole of the plantlet by their greater length, higher degree of vacuolation, smaller number of phaeoplasts, and less dense contents. By divisions, at right angles to the first division of the tetraspore, in both planes, a tuber-like structure is now formed (Pl. VI, Fig. 8). This was described and illustrated

by Reinke for *Taonia*, and is similar to the so-called central nodule in *P. pavonia* and *Haliseris* (*Dictyopteris*) also first described by Reinke. It may segment into relatively few cells, as in Pl. VI, Figs. 7 and 8, before giving rise to a projecting cell with denser contents from which the new plantlet arises. This cell may arise either terminally or laterally from near the pole of the primary tubercle opposite to the first rhizoid of the latter. Definite evidence has repeatedly been obtained, from the orientation of the rudimentary plants arising on the primary tubercles in the Petri-dish cultures, that the direction of the incident light determines the point of origin of the cell which is to give rise to the plantlet (Pl. VI, Fig. 7). It is noteworthy, however, that the new plant invariably arises on the primary tubercle on the side remote from the first rhizoid. A survey of the figures will make this clear. This fact appears to suggest that there is a polarity which is determined earlier, at the time of the first division of the germinating tetraspore. If this is the case it also appears likely that, as in the case of the fertilized eggs of *Fucus*, *Cystoseira* and other members of the *Fucaceae*, the direction of the incident rays of light is the factor determining the initial polarity, before the first division of the tetraspore occurs on germination.

The germination of the spherical undivided bodies which are discharged from the tetrasporangium differs from that of the tetraspore.

Segmentation takes place in a somewhat irregular manner, producing a tubercle of 8–16 cells (Pl. VI, Figs. 9–12). Subsequent behaviour varies in culture to some extent. Rhizoids may originate from one or more of the peripheral cells at one pole of the tubercle (Pl. VI, Fig. 11), while a peripheral cell at the opposite pole may give rise to a thallus (Pl. VI, Figs. 9, 10, 12). It would seem that here, as in the tetraspore, the initial polarity is determined by the direction of incident light.

Again, presumably when cultural conditions are unfavourable, from several peripheral cells of the tubercle, rhizoid-like filaments grow out which have the potentiality of forming thalli by apical segmentation when conditions improve (Pl. VI, Fig. 14).

In this case many separate thalli may be formed from one tubercle, while in the case first cited one thallus is produced (Pl. VI, Fig. 16). Pl. VI, Fig. 17 shows an abnormal case where two thalli have been produced from the opposite poles of the primary tubercle. There has been no indication of primary initial segmentation into four cells and the subsequent formation of both rhizoid and thallus from one quadrant. Such a phenomenon might have suggested that the discharged contents of the tetrasporangium comprised four tetraspores which have remained united. Material has been fixed, and it is hoped that a cytological investigation may reveal the nuclear condition of the unsegmented tetrasporangium. It is conceivable that, as *Taonia* is near its northern limit in Cardigan Bay, the

energy necessary for division of all the tetrasporangia is lacking. An attempt was made to discover whether the liberation of undivided cells was greater when the thalli had passed maturity, but no such indication was obtained.

Cultural work has indicated that the undivided contents possess far greater powers of resistance and germination than the tetraspores themselves. Furthermore, the resulting plantlets are larger and show more vigorous growth. It is possible, therefore, that they may be more effectual in reproducing the plant. If the contents of the undivided tetrasporangium contain a  $2 \times$  nucleus on germination, it is possible that such a means of reproduction explains the frequency of tetrasporic plants, as compared to sexual plants, in this locality.

#### SUMMARY.

1. In certain localities for *Dictyota* and *Padina*, and in most localities for *Taonia*, full development of the sexual plants arising from tetraspores is inhibited.
2. There is apparently a rhythmic production of bands of hairs and tetrasporangia at the apex of the thallus of *Taonia atomaria*, but subsequently intermediate cells of the thallus may become tetraspore mother-cells.
3. The daily tidal rhythm and the production of tetrasporangial bands appear to be correlated. Rhythmic differences in the density of sporangia in adjoining zones suggest a correlation with the different amounts of light received by the apical margin in the inter-tidal periods.
4. Germlings collected at Aberystwyth adhering to old thalli appeared to be of two kinds, and were subsequently found to have arisen from single tetraspores in the one case and from the germinated undivided contents of the tetrasporangium in the other.
5. Germination of both types was studied in culture.
6. The greater vigour of the plants derived from the undivided contents of the tetrasporangium suggests that this means of reproduction may explain the predominance in nature of the tetrasporic plants.

BOTANICAL DEPARTMENT,  
ABERYSTWYTH.

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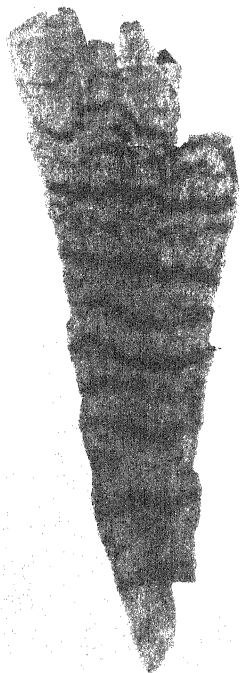
## EXPLANATION OF PLATE VI.

Illustrating Dr. Wilfrid Robinson's paper, Observations on the Development of  
*Taonia atomaria*, Ag.

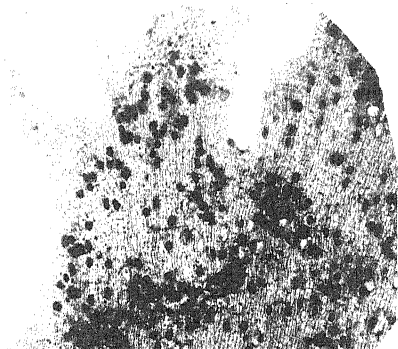
### PLATE VI.

- Fig. 1. Portion of thallus showing sporangial zones.  $\times 2$ .
- Fig. 2. Apical portion of a thallus bearing scattered sporangia.  $\times 20$ .
- Fig. 3. Two well-marked sporangial zones between which a secondary zone is developing.  $\times 20$ .
- Fig. 4. Division of some of the tetraspore mother-cells into two daughter-cells.  $\times 120$ .
- Fig. 5. Portion of thallus showing tetrasporangia, some of which have liberated their contents.  $\times 75$ .
- Fig. 6. Germinating tetraspore with a rhizoid growing from the basal cell.  $\times 75$ .
- Fig. 7. Group of germlings developed from tetraspores. The multicellular thalloid expansions and the septated filamentous rhizoids have arisen at opposite poles of the primary tuber-like structure.  $\times 75$ .
- Fig. 7a. Single germinating tetraspore.  $\times 75$ .
- Fig. 8. Germinating tetraspore showing development of primary tubercle.  $\times 130$ .
- Fig. 9. Germinating tetraspore mother-cell with two rhizoids. The thallus is growing out as a filament from the opposite pole of the primary tubercle.  $\times 130$ .
- Fig. 10. Germinating tetraspore mother-cell consisting of an irregularly segmented primary tubercle from one pole of which have arisen two rhizoids, and from the opposite pole a thallus which was at first filamentous and later formed a flat expansion by segmentation at the apical portion.  $\times 130$ .
- Figs. 11 and 12. Germinating tetraspore mother-cells. In the primary tubercle a peripheral region can be distinguished from the central region.  $\times 130$ . Fig. 11. Three rhizoids are developing at the basal region. Fig. 12. Rhizoid and thallus are developing at opposite poles.
- Fig. 13. Germinating tetraspore mother-cell consisting of a 2-lobed primary tubercle (?), a well-developed thallus, and a primary rhizoid.  $\times 130$ .
- Fig. 14. Germinating tetraspore mother-cell. Segmentation of the primary tubercle very irregular. Note outgrowth of peripheral cells in all directions. Thallus well developed.  $\times 130$ .
- Fig. 15. Group of germlings derived from tetraspores. Apical meristem consists of a marginal row of cells.  $\times 75$ .
- Fig. 16. Development of a thallus at the apical portion of a filamentous outgrowth from the primary tubercle of a tetraspore mother-cell. Note the presence of other similar outgrowths.  $\times 75$ .
- Fig. 17. Large plantlet derived from a tetraspore mother-cell, showing apical margin meristem. A second plantlet is developing at the opposite pole near the point of origin of the rhizoids.  $\times 130$ .

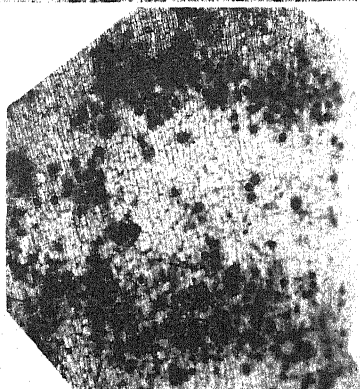




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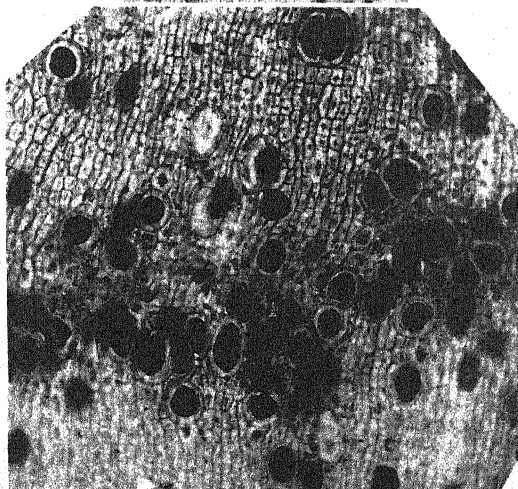
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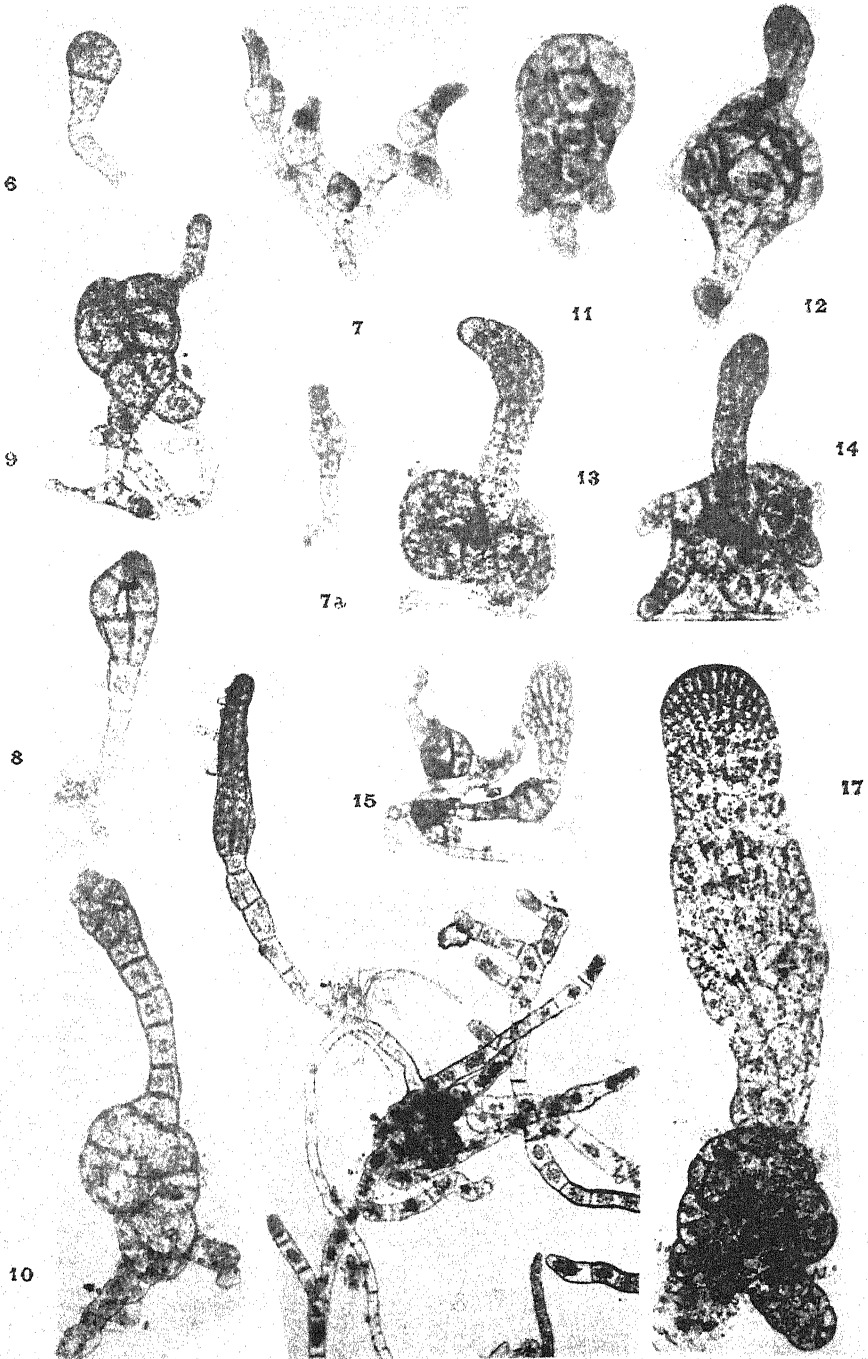
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# A Quantitative Study of the Geotropism of Seedlings with Special Reference to the Nature and Develop- ment of their Statolith Apparatus.

BY

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With fifteen Figures in the Text.

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## I. INTRODUCTION.

ALTHOUGH a large amount of work has been published on the geotropism of seedlings, the morphology of the statolith apparatus has received very little attention, and it was suggested to me by Dr. T. L. Prankerdt that an investigation of the relation between graviperception and the presence of statoliths in seedlings would probably yield interesting results. Quantitative work has been recorded on the geotropism of a few species, Czapek (5), Fitting (7), Bach (1), Brain (3) and others having worked out the geotropic presentation time and latent time of various seedling organs. Bach also gives the results of one series of experiments showing that P.T. and L.T. are dependent on the length of the organ studied in the case of the epicotyl of *Vicia Faba*. Apart from this, no previous

attempt has been made to work out 'graviscrits'<sup>1</sup> for presentation time and latent time for seedling organs, although detailed graviscrits have been given for fern fronds by Waight (32) and Prankerd (20). No quantitative work has previously been attempted on the statolith apparatus itself, although Prankerd (18) pointed out that the amount of statenchyma (21), or statolith-containing tissue, present in fronds of *Asplenium bulbiferum*, appeared to vary with the changes in geotropic sensitivity, and Herzog (10) gave a qualitative account of the correlation between the position of the statolith-containing tissue and the zone of geotropic sensitivity in certain seedlings.

From a consideration of the present position of the statolith theory, it is clear that further observations of a merely qualitative nature have little to add to the evidence in support of the theory, and an attempt has been made in the present work to supply more exact quantitative data.

## 2. EXPERIMENTAL METHODS.

All the geotropism experiments, unless otherwise stated, were conducted in a greenhouse at a temperature of  $20^{\circ} \pm 1^{\circ}$  C. and in an atmosphere of 70–80 per cent. humidity. The seeds were sown in pots or small boxes and, in all cases, were germinated and grown on in the greenhouse in which the experiments were performed.

The upright method (Waight (32)) was used in all experiments, the organ to be studied being placed in a horizontal position for the desired period of stimulation and then replaced in an upright position. This method allows the use of larger numbers of seedlings than the klinostat method (in which the seedlings are rotated on a klinostat after stimulation) and has been shown by Waight (32) and Brain (3) to give similar results. When necessary, the seedlings were supported on white cotton wool during long periods of stimulation to prevent sagging.

The presentation time (which will be referred to as P.T.) has been taken to be the period of stimulation which is necessary to produce a curvature of about  $5^{\circ}$  (rarely exceeding  $10^{\circ}$ ) in 75 per cent. of the seedlings used (Brain (3)). Latent time has been taken as the period from the beginning of stimulation to the first visible appearance of response and will be referred to as L.T. Table I illustrates the method by which values for P.T. were determined in a typical series of experiments.

In order to examine the statolith-containing tissue (which comprises the statolith apparatus), seedlings were stimulated for 30 minutes (or more where the P.T. was very long) with the organ to be studied in a horizontal

<sup>1</sup> A graviscrit has been defined by Prankerd (20) as a curve 'where the ordinates are the presentation times' (or latent times), and the abscissae the periods intervening between the stages. Stage of development is represented in the present work by length of the seedling organ to be studied.

position, but without removing the seedlings from the boxes in which they were grown. The seedlings were then fixed in a horizontal position in acetic alcohol (1 part glacial acetic acid to 3 parts absolute alcohol). After 24 hours they were transferred to absolute alcohol, then to 95 per cent. alcohol, and finally to 70 per cent. alcohol in which they were stored.

TABLE I.  
*Lathyrus odoratus*, epicotyl.

Height of epicotyl in cms.	Period of stimulation in min.	No. of seedlings used.	No. of seedlings curved 5° or more.	% age curved.	Deduced P.T. in min.	Average L.T. in min.
0-1	34	12	12	100	—	—
	20	15	12	80	20	45
1-2	34	12	12	100	—	—
	30	4	4	100	—	—
	20	19	18	94	—	—
	15	7	5	71.4	16	42
2-4	20	12	12	100	—	—
	15	12	10	83.3	—	—
	12	13	10	77	—	—
	10	20	10	50	12	39
4-6	11	7	6	84	—	—
	9	20	15	75	9	35
6-10	10	16	14	82	—	—
	8	33	26	79	8	35
10	12	37	27	75	—	—
	11	30	10	66	12	40

### 3. DICOTYLEDONS.

#### (a) *having hypogeal germination.*

In studying the geotropism of seedlings of the Dicotyledons, it is necessary to deal separately with those seedlings showing hypogeal and those showing epigeal germination, since in the former the hypocotyl remains short and the epicotyl<sup>1</sup> is the chief aerial organ of the seedling, while in the latter the hypocotyl becomes elongated and is strongly geotropic. It will be seen that with respect to geotropism, the epicotyl of the hypogeal forms and the hypocotyl of the epigeal forms are physiologically comparable to a certain extent, and more particularly in very young seedlings.

Among a number of species having hypogeal germination, detailed

<sup>1</sup> The term 'epicotyl' is here used to denote the first few internodes of the young shoot above the cotyledonary node.

graviscritps for geotropic P.T. and L.T. have been constructed for the epicotyl of *Lathyrus odoratus*. In very young seedlings of this plant, where the epicotyl is less than 1 cm. in length, the P.T. was found to be 20 min. falling, with increased growth in length of the epicotyl, to 8 min. for epicotyls of length 6–10 cm. and then rising to 12 min. for epicotyls of 10 cm. and over, thereafter remaining fairly constant during the elongation of the next few internodes. Values for L.T. were also determined. The values of P.T. and L.T. are given in the first three columns of Table II and are shown graphically in Fig. 1, where the data for P.T. and L.T. is plotted against length of epicotyl. It can be seen that the graviscritps for P.T. and L.T. are more or less parallel curves, the minimum values occurring at the same stage for both P.T. and L.T.

TABLE II.  
*Lathyrus odoratus*, epicotyl.

Length of epicotyl in cm.	P.T. in min.	L.T. in min.	Relative sensitivity to gravity.	Volume of statenchyma in epicotyl in cubic mm.	Rate of fall of statoliths in microns per hr.	Rate of growth of epicotyl in cm. per day.
0–1	20	45	5	—	—	0.3
1–2	16	42	6	(1.8) <sup>1</sup> 0.955	—	1.3
2–4	12	39	8	—	36	1.7
4–6	9	35	11	(4.7) 2.473	46	1.7
6–8	8	35	12.5	(6.2) 2.99	54	1.8
				(7.6) 3.629		
8–10	8	35	12.5	(9.1) 2.978	54	1.8
10+	12	40	8	(11.4) 2.645	46	1.8
				(16.9) 2.274		
				Significant Difference		
				0.755		

It was thought possible that the rise in P.T. and L.T. in epicotyls over 10 cm. long might be due to the injurious effect of the high temperature, 20° C., at which they were grown, but experiments at a temperature of 15° C. proved that this was not the case, since a similar rise was noted at the lower temperature.

It will be seen from Table II that, although the rate of growth increases with the initial decrease in P.T., there is no subsequent fall in growth rate to correspond with the rise in P.T.

Experiments on the primary root of *L. odoratus* show that the minimum P.T. of the root is much higher than that of the epicotyl. The L.T. is also longer, although it has not been worked out accurately. This

<sup>1</sup> The figures in brackets represent the actual mean lengths of the epicotyls in the samples used.

fact is of interest, since from a consideration of the environment of root and shoot, it is apparent that the former has not the same need for rapid orientation. The variations in sensitivity in the root simply follow growth

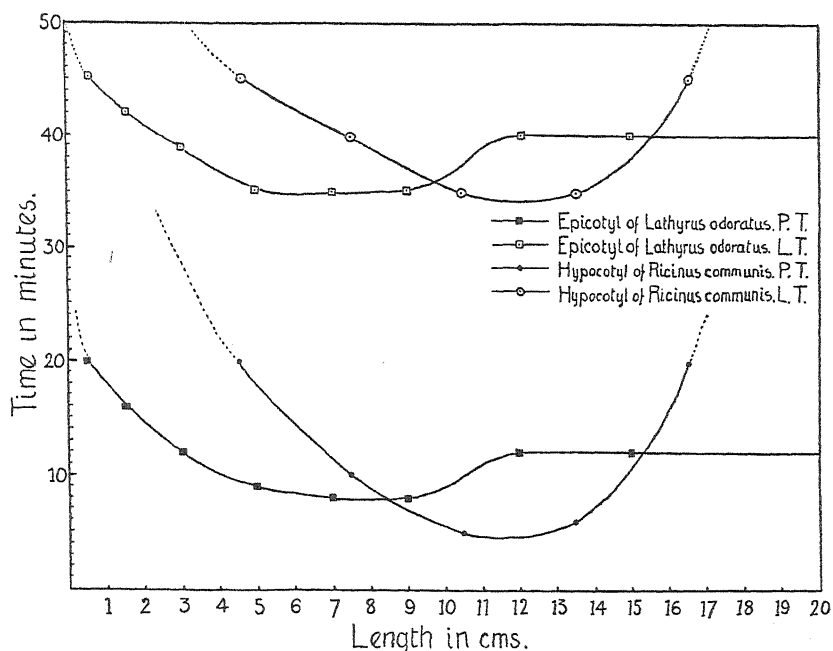


FIG. 1. Graviscritps for presentation time and latent time for the epicotyl of *Lathyrus odoratus* and the hypocotyl of *Ricinus communis*.

rate, hence, very little work has been done on roots in this case, the aerial organs of the seedling proving of greater interest. The results obtained for roots are shown in Table III.

TABLE III.

P.T. for Primary Roots.

Length of root in cm.	<i>Lathyrus odoratus</i> P.T. in min.	<i>Helianthus annuus</i> P.T. in min.	<i>Cucurbita Pepo</i> P.T. in min.
0-2	60	40	30
2-4	30	25	20
4-6	25	20	18
6+	25	20	18

On examination of a seed which has been soaked for 24 hours, the cells of the cotyledons are seen to be closely packed with starch grains of large size (their average diameter being about  $25\mu$ ). In the other parts of the embryo the starch grains are much smaller ( $2-3\mu$  in diameter). They are found in the ground tissue of the radicle and in the cells of the

pith and cortex of the plumule. This non-movable starch may conveniently be termed embedded starch to distinguish it from statolith starch. When germination begins, statolith starch first appears in the columella of the root cap of the radicle. In a very young seedling, in which the plumule is only 2 mm. long, the small grains of the embedded starch are particularly concentrated in the endodermis and in the single layer surrounding the four leaf traces which occupy the angles of the epicotyl as seen in T.S., but no free-falling or statholith starch is seen. In a slightly older seedling, where the plumule is 3-4 mm. long, some of the starch grains, in the endodermis and in the layer surrounding the leaf traces, are free to fall, although still very minute. Falling starch grains are also seen on the outer side of the vascular strands which pass into the cotyledons.

The amount of embedded starch decreases with the growth of the epicotyl, until, in those 6 cm. long or more, it is confined to the apical bud and to the apical part of the epicotyl. Fig. 2 illustrates the distribution of starch in an epicotyl 7.5 cm. long. In the apical part (about 1 cm. in length), although the endodermal starch sheath is well developed, the statocytes<sup>1</sup> are not completely differentiated, some of the grains being free to fall and others remaining attached to the upper or lateral walls of the statocytes. This zone may be termed the *zone of development*. Statocytes from this region are illustrated in Fig. 2, D. The starch probably becomes free to fall by a decrease in the viscosity of the protoplasm.

Below this zone of development is a zone corresponding to the region of geotropic curvature, in which the statoliths have an almost diagrammatic appearance (Fig. 2, E) and which may be called the *zone of efficiency*. The statocytes are found in a single layer surrounding the vascular cylinder and the four leaf traces (Fig. 2, B). Sections through a leaf gap show that as the trace, with its abaxial strand of sclerenchyma, separates from an angle of the vascular strand the layer of statenchyma closes round it, leaving a gap in the endodermal sheath as well as in the vascular cylinder. The statolith sheath accompanies the leaf trace into the petiole and lower part of the lamina of the leaf (Fig. 2, C).

Below this zone is a third zone which I propose to call the *zone of disintegration*, since here the starch loses its power of movement and becomes embedded in the cytoplasmic lining of the statocyte, the compound grains becoming split up into smaller ones. The starch is then apparently used up for nutritive purposes, a function which Haberlandt (9) considers to be of secondary importance.

It is of interest that the viscosity of the protoplasm of the statocytes is apparently less than that of the adjacent cells, since in sections of fresh material, Brownian movement can be clearly seen in the statocytes but not in the cortical cells. This would seem to account for the fact that one of

<sup>1</sup> i. e. cells containing statoliths.

two adjacent cells may contain moveable starch and the other embedded starch. It also seems to indicate a degree of specialization in the endodermal starch sheath which it is difficult to explain otherwise than by the possession of a specialized function by the statoliths.

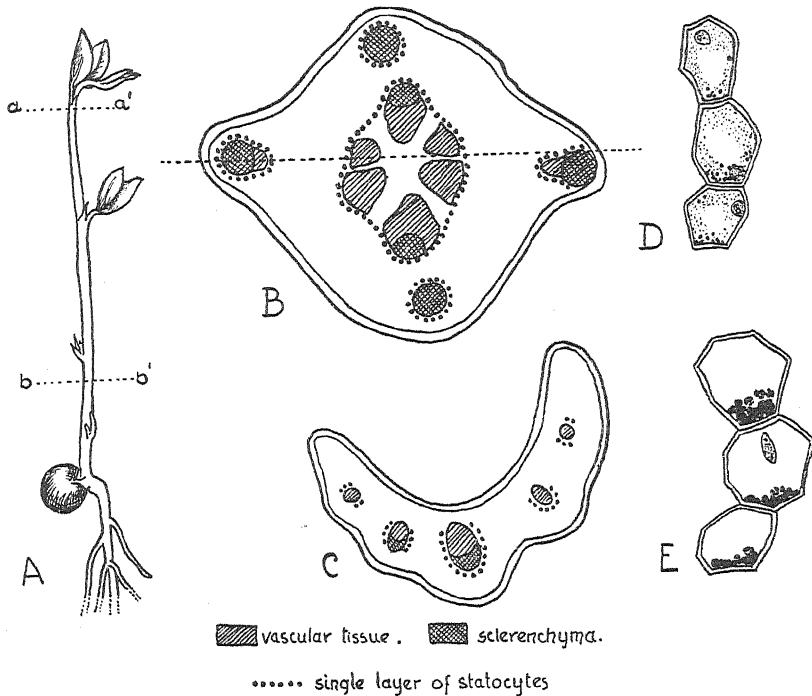


FIG. 2. A. Seedling of *Lathyrus odoratus*.  $\times \frac{3}{2}$ . Statoliths are found in the endodermis between planes  $a-a'$  and  $b-b'$ . B. Transverse section through plane halfway between  $a-a'$  and  $b-b'$  of A.  $\times 34$ . Dotted line represents cotyledonary plane. C. Transverse section of petiole.  $\times 34$ . D. Cells from endodermis in zone of development, showing incipient statoliths.  $\times 300$ . E. Cells from endodermis in zone of efficiency, showing fully developed statoliths.  $\times 300$ .

It seemed likely to be of interest to see if the correlation between statoliths and graviperception is as close in time as in space, and accordingly an attempt was made to estimate the amount of statenchyma present in epicotyls of different lengths. The area of the statenchyma seen in T.S. was calculated from the mean of camera lucida drawings, on squared paper, of transverse sections taken at the limits and at the middle of the zone of efficiency, and the results thus obtained were multiplied by the length of the zone of efficiency, giving the volume of statenchyma present. Following the adaptation of Fisher's (6) methods used by Mason and Maskell (12) for a series of observations in time, the whole of the data for a single series of calculations of the volume of statenchyma was pooled, and a significant difference was calculated for the series and plotted on the graph as a vertical line, to the same scale as that of the graph, for purposes of comparison.

The results obtained are shown in Table II and Fig. 3. From the significant difference plotted on the right, it can be seen that, although there is a fairly large sampling error, yet the changes in the amount of statenchyma are great enough to be regarded as significant.

On the same graph is plotted a curve obtained from the reciprocals of the P.T.'s. If, as first suggested by Waight (32), the sensitivity to gravity varies inversely with the P.T., then this curve may be taken to represent relative sensitivity to gravity. It can be seen from Fig. 3 that sensitivity to gravity is closely correlated with the volume of statenchyma present. That these are not merely parallel, unrelated growth phenomena, is shown by the fact that there is a much closer correlation between them than between either of them and rate of growth in length. Some variation in the amount of statenchyma present at any stage is found, but this is not very great, and this relative constancy is strongly contrasted with the large variation seen in the quantity of embedded starch present.

There are obviously many factors, in addition to the amount of statenchyma, which would influence the efficiency of the statolith apparatus, notably the size and number of the statoliths, the rate of fall of the grains (dependent on the viscosity of the protoplasm in the statocyte), the size of the statocyte (which would influence the time taken by the statoliths to reach the lower wall), and the sensitivity of the protoplasm on which the statoliths impinge.

In the first case, although it has not been found practicable to estimate, accurately, the size and number of the statoliths throughout the growth of the seedling, owing to the loosely compound nature and the heaping up of the grains, it is obvious, from an examination of a series of sections, that the bulk of the statoliths in the statocyte reaches a maximum in seedlings of 6-10 cm., i.e. of maximum sensitivity.

Of the factors mentioned above it would appear likely that the rate of fall of the statoliths would greatly influence the efficiency of the whole apparatus, and an attempt has been made to measure this rate. To do so, seedlings were first stimulated for thirty minutes, this time being known to be sufficient to allow the statoliths to become grouped on the lower walls of the statocytes. The seedlings were then rotated through an angle of  $180^\circ$  so that the statoliths would then be in a position underneath the roofs of the cells containing them. Thereafter samples were taken at varying periods in the new position and transverse sections cut and examined. In this way the time taken for the statoliths to fall through the distance of the cell diameter can be obtained, and from this data and the average diameter of the statocyte, as seen in T.S., the rate of fall of the statoliths can be calculated. The results obtained by this method are illustrated in Fig. 3 and are given in Table II. It will be seen that a correlation exists between the rate of fall of the statoliths and sensitivity to gravity,



although this is not so close as that between the latter and volume of statenchyma.

The average diameter of the statocyte as seen in T.S., remains fairly constant at  $27\ \mu$  throughout growth, and thus must have a fairly constant influence on the efficiency of the statolith apparatus.

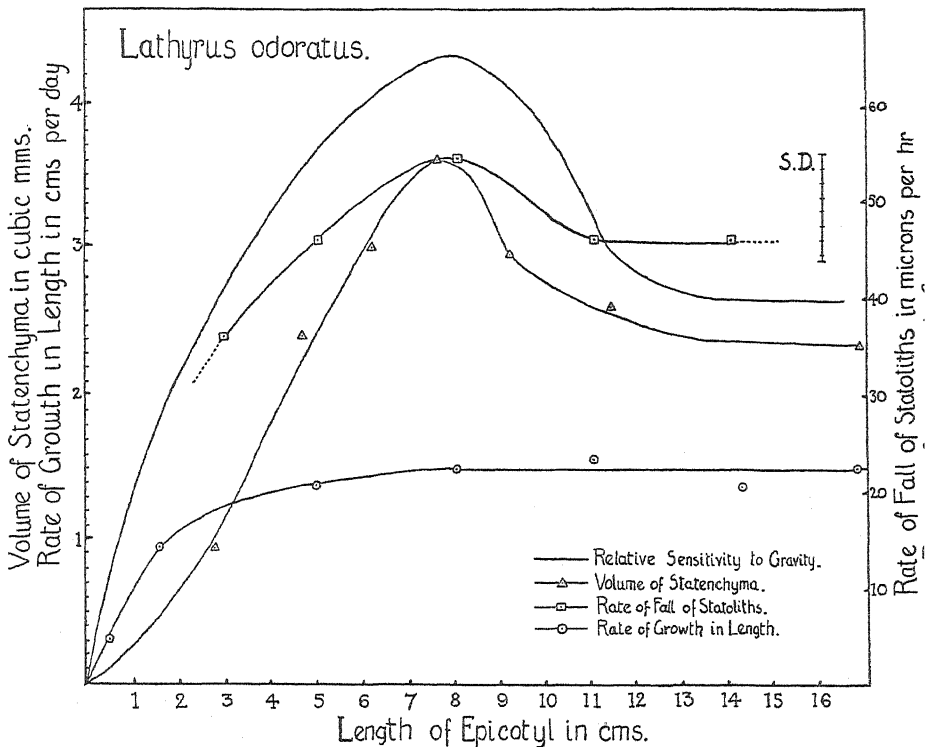


FIG. 3. *Lathyrus odoratus*. Graph to show relation between sensitivity to gravity, volume of statenchyma in the epicotyl, rate of fall of statoliths, and rate of growth in length of the epicotyl. S.D. represents significant difference for volume of statenchyma.

Unfortunately, in the present state of our knowledge, it is impossible to measure the sensitivity of the protoplasm, but the foregoing observations seem sufficient to indicate the existence of a close correlation between the efficiency of the statolith apparatus and sensitivity to gravity.

Seedlings of other hypogeal dicotyledons examined (i.e. *Vicia Faba*, *Aesculus hippocastanum*, *Quercus* sp., *Citrus paradisi*, and *Tropaeolum atropurpureum nanum*) are all essentially similar to that of *Lathyrus odoratus*, both in their response to gravity and in the morphology of their statolith apparatus, and hence the account given above for *L. odoratus* may be taken as typical of those dicotyledons which have hypogeal germination.

(b) *having epigeal germination.*

Sperlich (28), Tupper-Carey (31) and others have shown that the 'hook' or curvature of the tip of the hypocotyl, which is so conspicuous a feature of many young seedlings, is a positive geotropic curvature, and regard this as the first geotropic curvature of the hypocotyl. The advantage of this 'hook', as a device for the protection of the plumular bud during the emergence of the hypocotyl, is pointed out by Salisbury (27), who states that straightening of the 'hook' is more rapid under the influence of light, which agrees with the work of Priestley (24), who finds the 'hook' to be more pronounced in etiolated seedlings. Although it will be necessary to refer to this positive curvature from time to time, the present work is chiefly concerned with the more typical negative geotropism of the hypocotyl which persists long after the 'hook' has straightened out.

In order to give a clear idea of the geotropism and of the morphology of the statolith apparatus in the thirty species of epigeal dicotyledons studied, it will be necessary to describe several species in detail.

Brain (3), working with the hypocotyls of certain species of epigeal dicotyledons, pointed out that, in some cases, there is a difference in response to gravity between seedlings having a radially constructed hypocotyl and seedlings which she describes as 'zygomorphic'.<sup>1</sup> In the former P.T. and L.T. were found to be constant for stimulation in any plane, while in the latter both P.T. and L.T. were found to be shorter for stimulation in the cotyledonary plane than for stimulation in the intercotyledonary plane.<sup>2</sup>

Among seedlings with a radially constructed hypocotyl, *Ricinus communis*, on account of its size and well-developed statolith apparatus, is a convenient species for detailed study. The geotropic P.T. and L.T. of the hypocotyl are given by Brain (3) as 5 min. and 36 min. respectively, but a closer examination shows that, as in *Lathyrus*, there is a considerable variation in both P.T. and L.T. with growth in length. The nature of this variation is shown in Table IV and Fig. 1, and it will be seen that in both P.T. and L.T. there is first a fairly rapid fall, with increased growth rate, to a minimum value, followed by a rapid rise as rate of growth in length decreases. Before sensitivity is lost in the hypocotyl, the elongating epicotyl and the petioles of the cotyledons become sensitive to gravity and retain this sensitivity after all response has ceased in the hypocotyl.

<sup>1</sup> The term 'radially constructed hypocotyl' is used to denote one in which the vascular tissue, as seen in transverse section, is more or less circular in outline, whereas the so-called 'zygomorphic' type is one where the vascular tissue, as seen in transverse section, appears to be more or less elliptical in outline, i. e. the symmetry is bilateral.

<sup>2</sup> The cotyledonary plane has been defined by Thomas (30) as 'the plane joining the centres of two cotyledons', the intercotyledonary plane being the plane at right angles to this. Thus stimulation is in the cotyledonary plane when the hypocotyl is placed in a horizontal position with the cotyledonary plane vertical.

TABLE IV.

Length of hypocotyl in cm.	<i>Ricinus communis.</i>		<i>Cheiranthus Allionii.</i>		<i>Calendula officinalis.</i>		<i>Nigella damascena.</i>	
	P.T. in min.	L.T. in min.	P.T. in min.	L.T. in min.	P.T. in min.	L.T. in min.	P.T. in min.	L.T. in min.
0-1	—	—	20	60	15	70	18	55
1-2	—	—					11	50
2-3	—	—	7	40	12	60	12	57
3-4	20	45	6	40	11	60		
4-5			6	45	12	60+		
5-6			13	45+				
6-9	10	40						
9-12	5	35						
12-15	6	35						
15-18	20	45						
18+	20+	45+						

A distinct correlation can be traced between the geotropism and the distribution of statolith-containing tissue in both time and space in seedlings

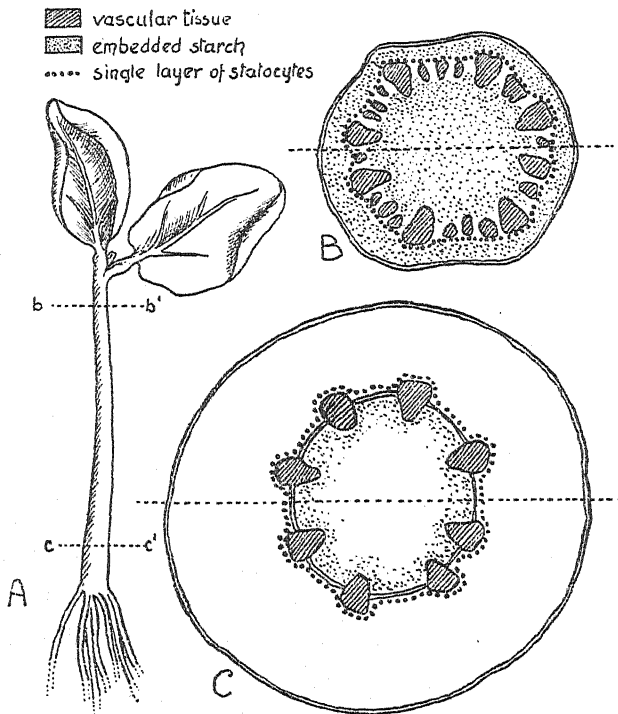


FIG. 4. A. Seedling of *Ricinus communis*.  $\times \frac{3}{2}$ . B, C. Transverse sections through planes b-b' and c-c' of A respectively.  $\times 12$ . Dotted line represents cotyledonary plane.

of *Ricinus*, although no quantitative work has been done on the statolith apparatus of this species.

In the very young seedling there is a large quantity of embedded starch in both cotyledons and hypocotyl, and statolith starch, not yet completely orientated, may be seen in the endodermis of the hypocotyl. The embedded starch decreases rapidly with the growth of the seedling, disappearing first from the base of the hypocotyl and remaining for some time in the apex. The distribution of starch in a seedling with a hypocotyl of 6 cm. length, is shown in Fig. 4. The statenchyma, which consists of a complete ring of cells (the endodermis), here shows zones of development, efficiency, and disintegration as in *Lathyrus*. With the growth in length of the hypocotyl there is an increasing part at the base of the hypocotyl which is neither sensitive to gravity nor contains statoliths. With the elongation of the epicotyl, statoliths and the power of graviperception are found in the epicotyl and in the cotyledon petioles, the zone of efficiency extending to the extreme apex of the hypocotyl. The statoliths disappear from the hypocotyl with the loss of geotropic sensitivity.

TABLE V.  
*Beta vulgaris*, hypocotyl.

Length of hypocotyl in cm.	P.T. in min.	L.T. in min.	Relative sensitivity to gravity.	Vol. of statenchyma in hypocotyl in cubic mm.
0-1	30	} 55 {	33	—
1-2	15		66	(1.4) <sup>1</sup> 0.209
2-3	10	46	100	(2.23) 0.422
3-4	15 +	57	66	(2.93) 0.255
				Significant Difference 0.0312

The volume of statenchyma present in the hypocotyl of *B. vulgaris* at different stages in its growth has been worked out by the method described above for *Lathyrus* (see Table V), and shows a very close correlation with the sensitivity to gravity as shown by the P.T. Czapek (5) has given 15 min. as the P.T. for the hypocotyl of *B. vulgaris*, and Table V shows that this is about the value which might be expected if no account were taken of change in P.T. during growth. It is of interest to find typical, well developed, starch statoliths in such a plant as *Beta*, where starch is not the usual product of photosynthesis, and it may be noted, that while the poorly developed embedded starch in the cortex of the hypocotyl disappears at a very early date, the statolith starch, in the endodermis, persists until growth in length ceases.

Other radially constructed seedlings for which graviscritps have been worked out are *Nigella damascena*, *Cheiranthus Allionii*, and *Calendula*

<sup>1</sup> The figures in brackets represent the actual mean length in cm. of the hypocotyls in the samples used.

*officinalis* (Table IV). The graviscritps for P.T. all show the same general shape as that for *Ricinus* (Fig. 1), i.e. a fall in P.T. to a minimum value, which varies with the species, and a subsequent rise, becoming steeper with decrease in growth rate. In a large number of other species, although graviscritps have not been fully worked out, sufficient work has been done to show that the curve would be similar in shape. Experiments performed on *C. Allionii*, *Capsella bursa pastoris*, and *Taraxacum officinalis* during June 1930, in the open, under natural conditions and with a temperature of approximately 20° C. confirm this.

It has already been mentioned that in the case of certain seedlings with non-radial hypocotyls, Brain (3) found a difference in P.T. and L.T. for stimulation in the cotyledonary and intercotyledonary planes. A study of the morphology of the statolith apparatus throws an interesting light on this phenomenon of bilateral symmetry in geotropism. Brain correlated this phenomenon with her so-called 'zygomorphic' type of hypocotyl. Since the statoliths are found in the endodermis or starch sheath of the hypocotyl, the more or less elliptical cross section of the vascular strand must result in an elliptical arrangement of the statenchyma as seen in T.S., and it might be thought that this alone would account for the difference in response for stimulation in the two planes. This, however, is not a satisfactory explanation, since in *Cucurbita Pepo* response is greatest in the direction of the short diameter of the hypocotyl and in *Lupinus polyphyllus* the reverse is the case.

A study of the statocytes themselves reveals the fact that these are not isodiametric in transverse section, which at once suggests a possible explanation of the geotropic bilateral symmetry. Considering an individual statocyte, it is obvious from Fig. 5, that, when stimulated in position *A*, less cell wall, with its sensitive layer of protoplasm, will be stimulated by the impact of the falling starch grains than in position *B*, and at the same time the statoliths will have a greater distance to fall and will thus take longer in reaching a position of equilibrium in position *A* than in *B*. Hence each statocyte will be more efficient in position *B* than in position *A* or any intermediate position. It is thus obvious, that if, for stimulation in one plane, there be more statocytes in position *B* than for stimulation in any other plane, a shorter period of stimulation would suffice to bring about response in that plane than in any other. Again, if the long diameter of the statocyte be inclined at an angle of between 30° and 60° to the cotyledonary plane, the statocyte will be equally efficient for stimulation in either the cotyledonary or the intercotyledonary plane (Fig. 5, C).

It is worth while to consider some of the seedlings which show bilateral symmetry, in some detail, when it will be found that the explanation suggested above can be applied in every case.

For hypocotyls of *C. Pepo*, Brain (3) gives 6 and 18 min. respectively

for P.T. for stimulation in the cotyledonary and intercotyledonary planes. My own results (given in Table VI) are slightly different, which may be due to the fact that Brain took no account of change in P.T. with growth,

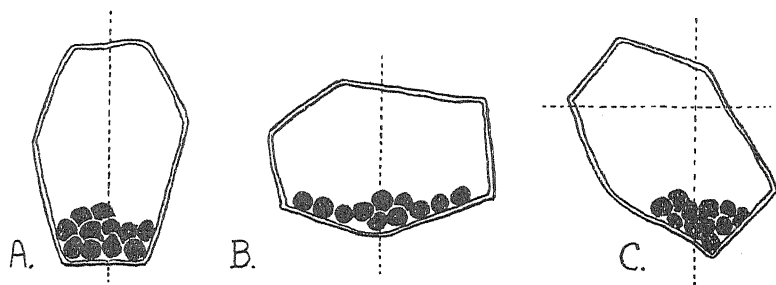


FIG. 5. A, B, C. A statocyte seen in transverse section after stimulation in various positions (diagrammatic). Explanation in text.

but used seedlings between 2 and 12.5 cm. long. It will be seen from Table VI that the P.T. shows a minimum value of 5 and 10 min. for stimulation in the two planes, in seedlings of 6–8 cm. length, and that the graviscrits for P.T. for either plane show a similar shape to that of the graviscrit for *Ricinus*. Graviscrits for L.T. are seen to be more or less parallel to those for P.T. Results obtained for the geotropism of the radicle are shown in Table III.

TABLE VI.

*Cucurbita Pepo*, hypocotyl.

Length of hypocotyl in cm.	P.T. in min. for stimulation in cotyledonary plane.	P.T. in min. for stimulation in inter- cotyledonary plane.	L.T. in min. for stimulation in cotyledonary plane.	L.T. in min. for stimulation in inter- cotyledonary plane.	Relative sensitivity to gravity.	Volume of staten- chyma in cubic mm.	Rate of growth in cm. per day.
0.2	12	15	55	60	8	(1.0) <sup>1</sup> 1.275	—
—						(1.6) 1.98	1.33
2.4						(3.27) 3.77	2.07
4.6	6	12	45	55	16	—	2.07
6.8	5	10	43	50	20	(6.26) 8.5	1.8
8–10	6	12	43	50	16	(8.25) 9.5	1.07
10–12	10	15	45	55	10	(10.56) 6.412	0.7
12–15	15+	20+	50+	60+	6	(12.0) 3.07	—
						Significant Difference = 0.48	

The distribution of starch in *Cucurbita* is very similar to that in *Ricinus*. In the young seedling, when statoliths are still present in the

<sup>1</sup> Figures in brackets represent actual mean lengths of hypocotyls in samples used.

'peg' region, the statenchyma here forms a complete ring, but higher up the hypocotyl statoliths are only found in a single layer of cells placed abaxially to the vascular bundles, and not extending across the gaps between

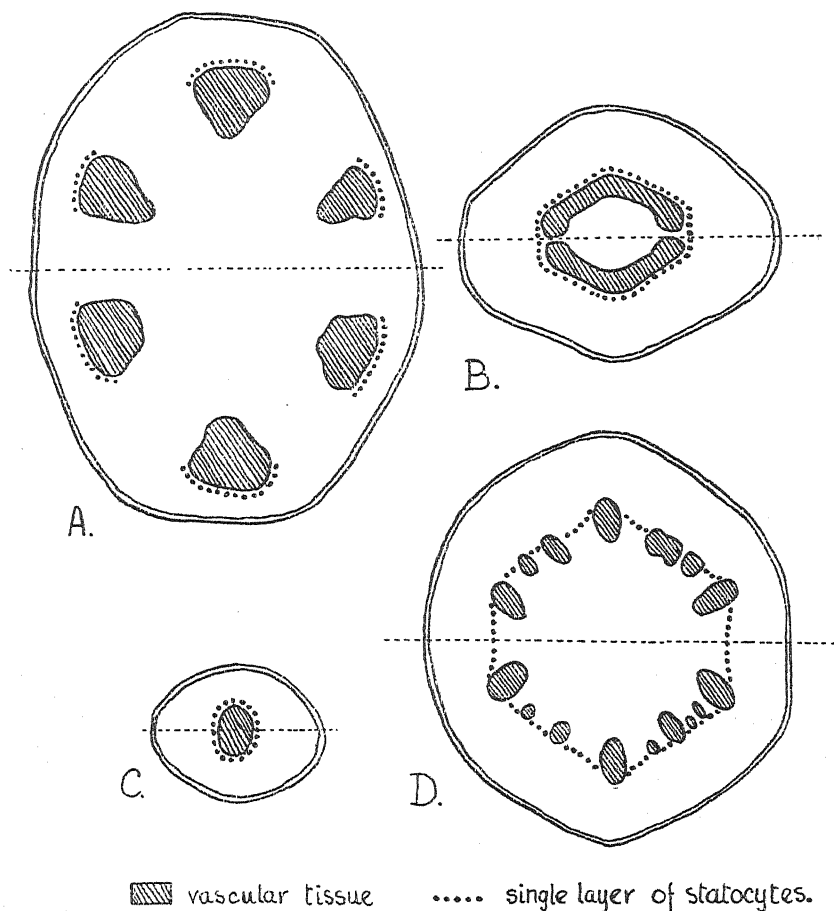


FIG. 6. A. Transverse section of hypocotyl of *Cucurbita Pepo*.  $\times 18$ . B. Transverse section of hypocotyl of *Lupinus polyphyllus*.  $\times 18$ . C. Transverse section of hypocotyl of *Aquilegia vulgaris*.  $\times 18$ . D. Transverse section of hypocotyl of *Helianthus annuus*.  $\times 18$ . Dotted lines represent cotyledonary plane.

the bundles (Fig. 6, A). The presence of statoliths in the 'peg' region of the young seedling indicates that gravity may, after all, play some part in 'peg' formation (4). It is of interest that there are occasionally a few statocytes placed adaxially to the bundles and apparently correlated with the presence of internal phloem. A quantitative study of the amount of statenchyma present during growth shows a much closer correlation (Table VI) between amount of statenchyma and sensitivity to gravity than between either of these and rate of growth in length.

An examination of the shape of the statocytes shows that these are

not isodiametric in transverse section, but are elongated tangentially. Thus, since the long axis of the hypocotyl as seen in T.S. is horizontal when stimulation takes place in the cotyledonary plane (Fig. 6, A), it follows that more statocytes will be in the most efficient position (B of Fig. 5) than when stimulation is in the intercotyledonary plane, which would explain the shorter P.T. for stimulation in the cotyledonary plane.

It is possible to obtain a rough mathematical ratio for statolith efficiency in the two planes. For seedlings of maximum sensitivity, i.e. having hypocotyls between 4 and 10 cm. in length, the ratio of the long and short diameters of the cross-section of the statocyte is 1 : 1.75, and the ratio of the number of statocytes with their long diameter horizontal when stimulation is in the cotyledonary plane to those with their short diameter horizontal is 1 : 66. Thus the ratio of the amount of statocyte wall stimulated when stimulation is in the cotyledonary plane to that when stimulation is in the intercotyledonary plane is

$$[(1.66 \times 1.75) + (1 \times 1)] : [(1 \times 1.66) + (1 \times 1.75)] :: 39 : 34.$$

But since the time for the majority of the statoliths to reach a position of equilibrium when stimulation is in the cotyledonary plane is 1.75 times that necessary for stimulation in the intercotyledonary plane, these two ratios may be multiplied together, giving a ratio of approximately 2 : 1 for *statolith efficiency* in the two planes, which corresponds to the ratio of P.T.'s.

It is of interest to note that in seedlings with hypocotyls less than 4 cm. or more than 10 cm. long, the ratio of P.T.'s for stimulation in the two planes is not so great as it is for seedlings of maximum sensitivity, and that the statocytes are more nearly isodiametric.

In *Cucumis sativus* the morphology of the statolith apparatus is similar to that of *Cucurbita*, and sufficient experiments have been performed to prove that this species also shows bilateral symmetry in the geotropism of the hypocotyl.

In *Lupinus polyphyllus*, for which Brain (3) obtained a ratio of 4 : 1 for P.T. for stimulation in the two planes, the statocytes are also elongated tangentially, but here the greater number are inclined at an angle of between 30° and 60° to the cotyledonary plane and are thus equally efficient in either plane. It will be seen from Fig. 6, B, that, as in *Cucurbita*, there is greater statolith efficiency when stimulation is in the cotyledonary plane. *Laburnum vulgaris* shows a similar arrangement of the statolith apparatus and is more sensitive when stimulated in the cotyledonary than in the intercotyledonary plane.

Experimenting with seedlings grown on the klinostat, Brain (3) found that the ratio of P.T. for stimulation in the two planes, for *L. polyphyllus*, hypocotyls, was reduced from 4 : 1 to 3 : 1, and pointed out that, while the



T.S. of the hypocotyl becomes circular and the cortical cells become more nearly spherical, the vascular strand retains its bilateral symmetry. An examination of the statocytes of such seedlings shows that these are more nearly isodiametric, which is in accordance with the fact that growth on the klinostat reduces the bilateral symmetry in the geotropism of the hypocotyl. In the hypocotyl of *Cucurbita Pepo*, growth on a klinostat does not alter the shape of the statocytes and the geotropic bilateral symmetry remains unaltered.

*Aquilegia vulgaris* is another species, for the hypocotyl of which, bilateral symmetry in geotropism has been recorded by Brain (8), the P.T.'s for stimulation in the cotyledonary and intercotyledonary planes being given as 35 and 45 min. respectively. In this seedling, although the long axis of the T.S. hypocotyl is in the cotyledonary plane, the long axis of the vascular cylinder, seen in T.S., is in the intercotyledonary plane (Fig. 6, C). The statocytes are elongated tangentially and hence there is greater statolith efficiency when stimulation is in the cotyledonary than in the intercotyledonary plane. The statoliths are also found in the fused bases of the cotyledon petioles, and here, too, the statocytes are elongated tangentially. It is of interest that in this case, where the ratio of the P.T.'s for the two planes is small, the statocytes are nearly isodiametric in transverse section.

The case of *Helianthus annuus* is of special interest from the point of view of the geotropism of the hypocotyl. It was described by Brain (8) as one of her 'zygomorphic species', in which the P.T. was less for stimulation in the cotyledonary than in the intercotyledonary plane.<sup>1</sup> On repeating her experiments on *Helianthus* it soon became evident that this was not always true, but that, in some seedlings, *the reverse was actually the case*, i.e. the P.T. was less for stimulation in the intercotyledonary plane than for stimulation in the cotyledonary plane.

The statolith apparatus in the hypocotyl consists of a single row of statocytes, but these only extend across the gaps between the vascular strands and are not found placed abaxially to the strands (Fig. 6, D). In seedlings which are known to be more sensitive to stimulation in the cotyledonary plane, the statocytes are found to be elongated tangentially, which would give greater statolith efficiency in the cotyledonary plane (Fig. 6, D), while in seedlings known to be more sensitive when stimulated in the intercotyledonary plane, the statocytes are elongated radially, giving greater statolith efficiency in the intercotyledonary plane.

The volume of statenchyma present in the hypocotyl of *H. annuus* during growth shows the usual close correlation with geotropic sensitivity (Table VII).

<sup>1</sup> The transverse section *Helianthus annuus* hypocotyl in Text-fig. 2a of Brain's paper is wrongly oriented, the cotyledonary plane being 'horizontal' and not 'vertical' in this case.

It is of interest that species which have a non-radial vascular cylinder, but which possess statocytes which are isodiametric in T.S. (e.g. the epicotyl of *L. odoratus*), do not show bilateral symmetry in geotropism, indicating that this phenomenon is not due solely to the non-radial construction of the hypocotyl or epicotyl.

It may also be noted here that the shape of the statocytes will provide an explanation of the fact recorded by Brain (3), that a higher percentage of response to gravity is shown in the direction of the long diameter of the cross-section of the coleoptile of *Avena sativa*.

TABLE VII.

*Helianthus annuus*, hypocotyl.

Length of hypocotyl in cm.	P.T. in min.	L.T. in min.	Relative sensitivity to gravity.	Volume of statenchyma in hypocotyl in cubic mm.	Rate of growth in length in cm. per day.
0-2	20	63	50	—	—
2-4	10	57	100	(2.3) <sup>1</sup> 1.785	1.1
—	—	—	—	(3.1) 2.166	—
4-6	5	52	200	(4.0) 2.99	1.3
—	—	—	—	(5.5) 3.508	—
6-8	6	52	166	(6.26) 3.47	0.83
8-10	10	67	100	(8.1) 1.605	0.5
				(9.06) 0.97	—
				Significant Difference = 0.42	

It is now possible to give a general account of the geotropism and the morphology of the statolith apparatus in seedlings of epigeal dicotyledons.

The radicle is the first part of the seedling to become geotropic, when it increases in sensitivity with increased growth rate, the P.T. finally reaching a minimum value at which it then remains.

The first geotropic curvature in the hypocotyl is the positive curvature of the 'hook' region. Before the hook is straightened out, the region behind the hook becomes negatively geotropic, and this more typical negative geotropism persists long after the positive curvature has disappeared. In all species examined, both P.T. and L.T. decrease with growth in length of the hypocotyl and then increase rapidly as growth ceases, the epicotyl then becoming geotropic.

It has been suggested (21) that typical statoliths are not present in very delicate organs such as the extremely slender hypocotyls of some seedlings. An investigation of several species with slender hypocotyls has proved that this is not the case, since in all seedlings examined the presence of statoliths could be demonstrated by suitable methods. In

<sup>1</sup> Figures in brackets represent the actual mean lengths of the hypocotyls in the samples used.

many cases it is practically impossible to obtain an undamaged hand section of an extremely slender hypocotyl and, in this case, some other method must be used to supplement the ordinary method of hand-sectioning. For several reasons the microtome cannot be used, the chief objection being the tendency of the starch grains to become washed out during the process. The statoliths can, however, be easily demonstrated if the seedlings are fixed in an upright position (without previous stimulation in a horizontal position) and dissected in a drop of iodine. It is usually possible to dissect away the epidermis and cortex of the hypocotyl, leaving the vascular cylinder surrounded by the endodermis, in which the statoliths can be clearly seen lying in heaps on the lower walls of the cells. By this means the presence of statoliths can be demonstrated in the endodermis of such slender hypocotyls as those of *Daucus Carota* (1 mm. diameter), *Apium graveolens* (0.3 mm.), *Anemone* sp. (0.5 mm.), *Convolvulus sepium* (0.5 mm.), *Salix* sp. (0.5 mm.), and *Nicotiana Tabacum* (0.25 mm.). Statoliths can also be demonstrated by this method in the hypocotyl of the aquatic *Hottonia palustris* (0.3 mm.), which although known to be geotropic (16), has not previously been shown to possess statoliths. The sizes of the statolith and statocyte in these slender species do not differ greatly from those in larger seedlings, examples of which are shown in Table VIII.

TABLE VIII.

Species.	Minimum P.T. in min.	Average diameter of statocyte in microns.	Average diameter of statolith in microns.	Average diameter of embedded starch grain in microns.
<i>Ricinus communis</i> . . .	5	20.5	10	4
<i>Cheiranthus Allionii</i> . . .	6	26.8	5	2
<i>Lathyrus odoratus</i> . . .	8	27	6	3
<i>Beta vulgaris</i> . . . . .	10	26.5	3	2.5
<i>Nigella damascena</i> . . . .	11	31.8	7	3
<i>Calendula officinalis</i> . . .	11	19	6	3

In addition to the species already mentioned, the statolith apparatus has been examined in the following: *Ranunculus aquatilis*, *Reseda odorata*, *Alyssum maritimum nanum*, *Raphanus sativus*, *Pyrus malus*, *Crataegus Oxyacantha*, *Gypsophila elegans*, *Beta* sp., *Euphorbia Peplus*, *Acer Pseudoplatanus*, *Carpinus Betulus*, *Fagus sylvatica*, *Asclepias curassavica*, *Myosotis palustris*, *Solanum capsicastrum*, *S. Lycopersicum*, *Physalis Alkekengi*, *Schizanthus* sp., *Galium Aparine*, *Scabiosa atropurpureum*, *Callistephus* sp., *Lactuca scariola*, and *Cosmea* sp., making 42 species drawn from a wide variety of families. From a consideration of these species, it becomes evident that the morphology of the statolith apparatus is essentially uniform in the hypocotyl of the dicotyledonous seedling, consisting typically of a single layer of cells, the endodermis, surrounding the vascular cylinder,

e.g. *Ricinus communis*. There are a few slight variations from this usual arrangement. In *C. Pepo*, as already noted, the statenchyma does not extend across the gaps in the ring of vascular bundles, except in the 'peg' region, while in *H. annuus* the opposite is the case, the statocytes having a sort of 'chain' formation linking the vascular bundles, but not occurring opposite the bundles themselves, and in *Euphorbia Peplus* the ring of statocytes is not complete. In a few arboreal species, e.g. *Crataegus*, *Fagus*, &c., two or more rows of statocytes are found.

#### 4. MONOCOTYLEDONS.

Owing to the difficulty of germinating seeds of some monocotyledons, the number of species studied (16 in all) was not so great as could be desired. In many cases it was found that the use of the ether method, described by Rayner (25) for *Vaccinium*, was of considerable help in speeding up germination. With the exception of the grass coleoptile, very little is known about the geotropism of monocotyledonous seedlings, which is the more surprising, since these seedlings are of considerable interest from this point of view.

Four species, *Allium Cepa*, *Canna indica*, *Commelina coelestis*, and *Asparagus officinalis* have been studied in detail.

Critical experiments on the geotropism of *A. Cepa* were discontinued after the emergence of the first foliage leaf from the cotyledonary sheath, hence the values given in Table IX are for the cotyledon alone. Measurements of geotropic curvature were made more difficult by the loop-like curve of the cotyledon during the earlier stages of development. Experiments on a klinostat show that this looping of the cotyledon and the subsequent straightening of the loop are not geotropic movements. The movements of the young cotyledon of *Allium* have been described in detail by Neubert (15), who states that the ultimate position of the cotyledon is the result of nutational and geotropic curvatures.

TABLE IX.

#### *Allium Cepa*, cotyledon.

Length of cotyledon in cm.	P.T. in min.	L.T. in min.	Relative sensitivity to gravity.	Vol. of statenchyma in cotyledon in cubic mm.
0-2	30	55	33	(1.0) <sup>1</sup> 0.12
2-4	19	45	52	(3.67) 0.162
4-6	12	42	83	(5.4) 0.24
6-8	6	40	166	(6.97) 0.242
8+	10+	65	100	(8.8) 0.11
				Significant Difference = 0.018

<sup>1</sup> Figures in brackets represent actual mean lengths of the cotyledons in the samples used.

The graviscrypt for P.T. for the cotyledon of *A. Ceba* shows an initial fall to a minimum value of 6 min. and a subsequent steep rise, and is similar to that of the dicotyledonous hypocotyl, with which the cotyledon of *Allium*

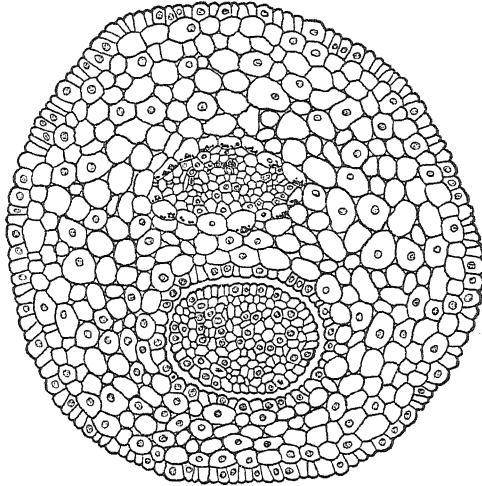


FIG. 7. Transverse section through base of young cotyledon of *Allium Ceba* showing position of statenchyma.  $\times 60$ .

is thus physiologically comparable. Experiments with *A. Porrum* and *A. ascolonicum* were sufficient to show both a general generic similarity and a distinct specific difference for the three species, thus providing an interesting point of comparison with the fronds of *Asplenium* and *Osmunda* which have been shown by Pranker (20) to show 'specificity in gravi-perception'.

The statolith apparatus of all three species of *Allium* examined is of considerable interest. It has long been known (Meyer (13)) that the leaves of *Allium* do not readily form starch as a product of photosynthesis, although Rendle (26) reports that a few small grains are sometimes present. Némec (14) has reported the presence of statoliths in the root-caps of *A. Ceba* and apparently considered these to be typical starch grains, while Haas and Hill (8) mention the presence, in the root-tip, of grains which, instead of giving the typical blue reaction with iodine, are stained a reddish brown, and which, according to them, consist of a mixture of dextrin and amylo-dextrin.

In the young cotyledon of *A. Ceba*, grains similar to those in the root-tip and of a statolith nature are found in a single layer surrounding the vascular bundle at the base of the cotyledon (Figs. 7 and 8). These disappear with the loss of geotropic sensitivity by the cotyledon and appear in the young foliage leaf on its emergence from the cotyledonary sheath. Némec (14) states that the nuclei of the statocytes of the root-tip arrange

themselves underneath the upper wall of the cell. The opposite is the case in the cotyledon, where the nucleus is always found at the bottom of the statocyte, often forming a typical 'nucleostatolith' (23) with the grains

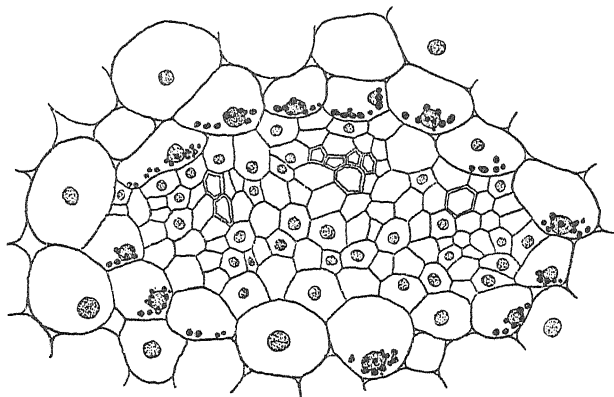


FIG. 8. Vascular strand of Fig. 7 enlarged.  $\times 180$ . Statoliths are shown in black.

attached to it. The statolith apparatus in *A. Porrum* and *A. ascolonicum* is of the same nature as that of *A. Cepa*.

An attempt was made to estimate the volume of statenchyma in the cotyledon of *A. Cepa* during growth, and the results show a close correlation with geotropic sensitivity (Table IX).

Thus although starch statoliths are by far the most common in plants, yet the plant is not dependent on the presence of true starch for the perception of gravity. The statolith of *Allium* provides an interesting modification, although not departing so far from the typical starch statolith as does the crystal statolith described by Pranker (17) for the wheat haulm.

In the typical monocotyledonous seedling, e.g. *Canna indica* and *Commelina coelestis*, the compact nature of the seedling makes it impossible to deal separately with the geotropism of the cotyledon and of the foliage leaves. Since the foliage leaves are usually closely packed within the cylindrical cotyledon, any curvature of the latter must necessarily cause curvature of the former. Moreover, the zone of curvature does not change with growth in length as in stem organs, but is always situated at the base of the leaves, where growth takes place. Thus the seedling curves as a whole in the region of the short, thick hypocotyl and the base of the cotyledon and foliage leaves.

In both *Canna indica* and *Commelina coelestis*, the graviscritps for P.T. show the usual shape (Table X), falling to a minimum of 18 min. for *Commelina*, but never falling below 27 min. for *Canna*, this comparatively high minimum P.T. being probably due to the slower growth and the more compact nature of the latter seedling. The L.T. is also longer than for most dicotyledons.

TABLE X.

Height of seedling in cm.	<i>Canna indica.</i>		<i>Commelina coelestis.</i>	
	P.T. in min.	L.T. in min.	P.T. in min.	L.T. in min.
0-1	120+	—	25	27
1-2	60	never	20	65
2-3	35	less	18	62
3-4	27	than	18	62
4-5	27	2 hrs.	21	70+
5-7	27			
7-9	40			

The statolith apparatus of these seedlings is correlated with the zone of geotropic curvature. Starch is present in large quantities in the endosperm of the seed of *C. coelestis* and is present in the embryo as soon as germination begins. Statolith starch grains of large size are present in the cotyledon on its emergence from the testa. The cotyledonary sheath reaches a length of about 1 cm. before the first leaf breaks through. At this point in the development of the seedling, statolith starch is distributed throughout the ground tissue of the base of the cotyledon, becoming less concentrated towards the apex, while in the extreme apex of the cotyledon, the statenchyma is limited to a single layer surrounding the vascular strands. There is a quantity of embedded starch in the young foliage leaf, the starch being particularly concentrated round the vascular bundles. Later, when the foliage leaf breaks through the cotyledonary sheath, statoliths are developed in the cells surrounding the vascular bundles (Figs. 9 and 10). As the cotyledon and the first foliage leaf cease to grow, the statoliths disappear and geotropic sensitivity is lost, statoliths being present only in the growing organs.

The statolith apparatus of *C. indica* is very similar to that of *Commelina*, but there is considerably less embedded starch present. The statenchyma does not usually form a complete ring surrounding the vascular bundles, but is placed laterally to the bundles.

The germination of *Asparagus officinalis* differs from the more usual type among monocotyledons, in the comparative unimportance of the cotyledon, which remains short and thick, and in the elongation of a slender epicotyl with long internodes. The cotyledon is not sensitive to gravity, and thus the values for P.T. and L.T. given in Table XI are worked out for the epicotyl. A comparison of Table XI and Table II shows an essential similarity between the geotropism of the monocotyledonous *Asparagus* and that of the dicotyledonous *Lathyrus*. Although the P.T. for *Asparagus* has a smaller range of values and a much lower minimum value than that for *Lathyrus*, there is the same initial fall, followed by a slight rise to a constant value. The minimum P.T. of 3 min. found for *A. officinalis* is the

lowest P.T. recorded for any *seedling* organ, although Brain (3) gives 3 min. as the P.T. for stimulation in the cotyledonary plane of the hypocotyl of *H. annuus*. Among Angiosperms certain inflorescence axes are equally

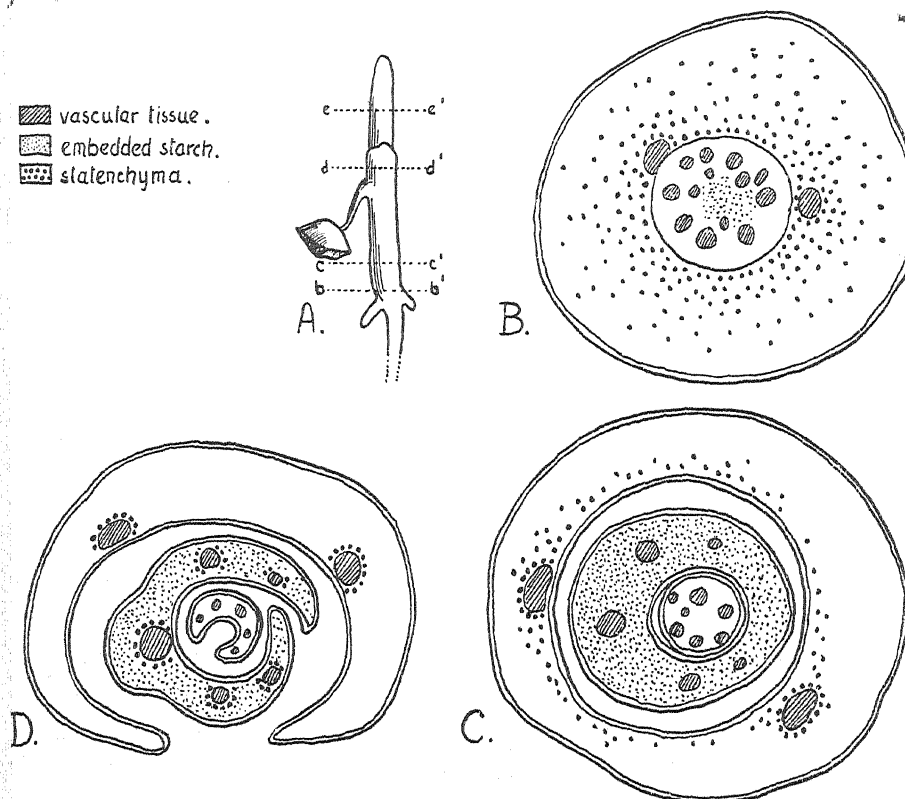


FIG. 9. A. Seedling of *Commelina coelestis*.  $\times \frac{3}{2}$ . B, C, D. Transverse sections through planes b-b', c-c', d-d' of A respectively.  $\times 45$ .

or more sensitive. Bach (1) reports less than 2 min. for the inflorescence axis of *Sisymbrium officinale*, *Plantago lanceolata* and *P. media*, but since he regards a stimulus sufficient to produce only 50 per cent. response as the P.T., these results are probably too low by the standard used here and are not directly comparable with my results. Much more sensitive organs have been found among ferns, Prankerd (20) reporting that at certain stages the fronds of *Osmunda regalis* and *O. cinnamomea* have a P.T. as low as 1 min. and 30 secs. respectively, while Streeter (29) obtained a response after only 1 min. stimulation with sporophores of *Amanita*.

Statoliths are never found in the ageotropic cotyledon of *Asparagus*, but are found in a single layer of cells surrounding the ring of vascular bundles (Fig. 11) in the epicotyl. In the young part of the epicotyl, the statenchyma shows zones of development, efficiency and disintegration,



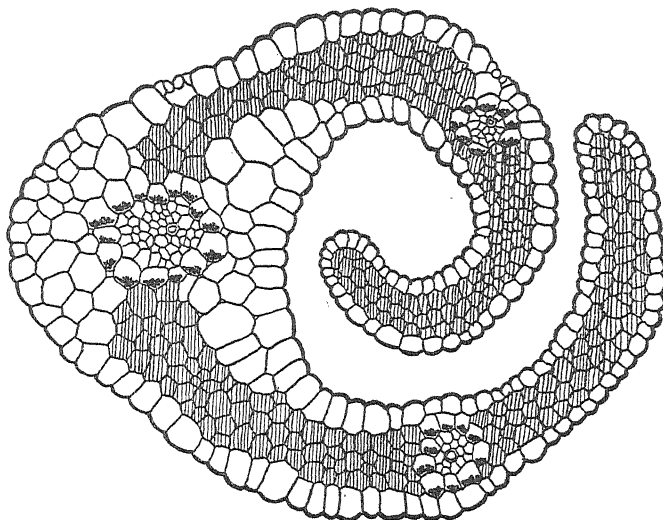


FIG. 10. Transverse section of first foliage leaf of *Commelina coelestis* through plane *e-e'* of Fig. 9, A.  $\times 100$ . Shaded area represents assimilating tissue. Statoliths are shown in black.

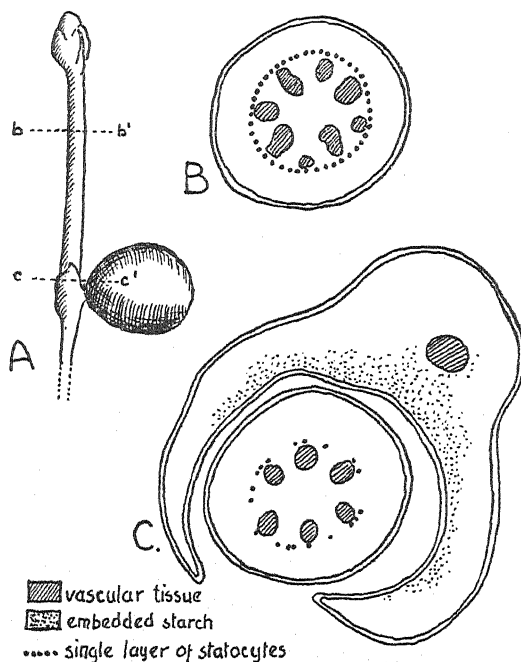


FIG. 11. A. Seedling of *Asparagus officinalis*.  $\times 2\frac{1}{2}$ . B, C. Transverse sections through planes *b-b'* and *c-c'* of A respectively.  $\times 34$ .

correlated with the zone of geotropic curvature as in *Lathyrus*. The statocytes of *Asparagus* are very small, averaging only  $19.8 \mu$  in diameter (Table XII), and it seems probable that this may be partly the cause of the low P.T., since the statoliths have only a short distance to fall. The statolith apparatus is similar in *A. Sprengeri* and *A. plumosus*. The volume of statenchyma present at different stages in the growth of the epicotyl of *A. officinalis* has been calculated by the method described for *Lathyrus*, and shows a close correlation with the sensitivity to gravity (Fig. 12 and Table XI).

TABLE XI.

Length of epicotyl in cm.	P.T. in min.	L.T. in min.	Relative sensitivity to gravity.	Volume of statenchyma in epicotyl in cubic mm.	Rate of growth in cm. per day.
0-1	10	67	10	—	0.5
1-2	7		12.5	—	0.5
2-4	4	50	25	(2.6) <sup>1</sup> 0.67	1.25
4-6	3	46	33	(4.4) 0.998	1.7
6-8	3	46	33	(7.3) 1.178	1.8
8-10	3	46	33	(9.26) 1.3	1.7
10	4	49	25	(12.8) 0.967	1.7
Significant Difference = 0.249					

A study of the morphology of the statolith apparatus has been extended to the following monocotyledons; *Alstroemeria haemantha*, *Amaryllis (Hippeastrum)*, *Asphodelus albus*, *Cordyline indivisa veitchii*, *Gladiolus* sp., *Iris pseudacorus*, *Scilla*, *Peruviana*, and *Phoenix dactylifera*. The distribution of both embedded and statolith starch in these species is, in general, similar to that described above for *Canna* and *Commelina* and the sizes of statolith and statocyte vary within about the same limits as those shown in Table XII.

TABLE XII.

Species.	Minimum P.T. in min.	Average diameter of statocyte in microns.	Average diameter of statoliths in microns.	Average diameter of embedded starch grains in microns.
<i>Allium Cefa</i> . . . . .	6	25	3.5	
<i>Asparagus officinalis</i> . . .	3	19.8	3	2
<i>Canna indica</i> . . . . .	27	33.5	3	2
<i>Commelina coelestis</i> . . .	18	35.8	8	} Seed 12.5 Leaf 1.5

<sup>1</sup> Figures in brackets represent the actual mean lengths of the epicotyls in the samples used.

*Alstroemeria*, *Asphodelus*, *Cordylina*, and *Scilla* have very little starch, the embedded starch being reduced to a minimum, usually being limited to the young parts of the seedling which have not yet formed statoliths.

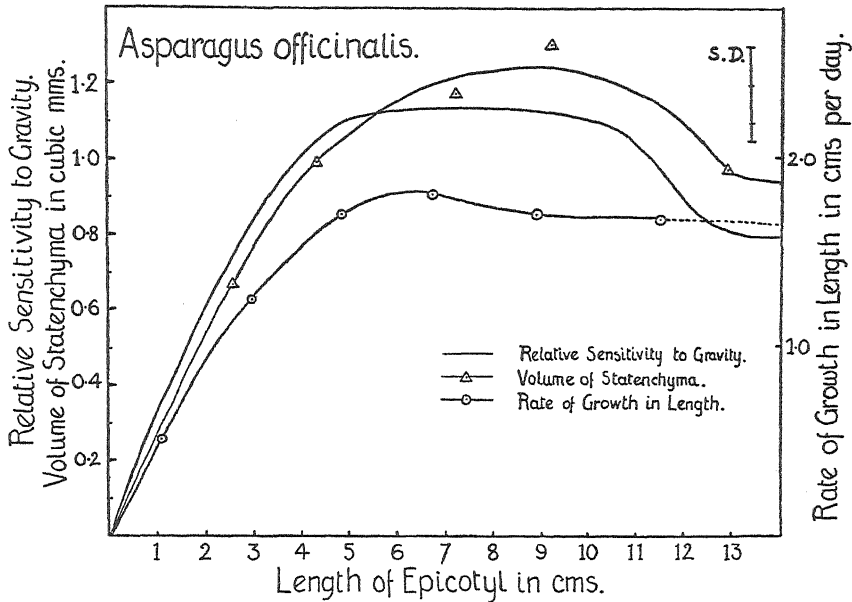


FIG. 12. *Asparagus officinalis*. Graph to show relation between sensitivity to gravity, volume of statenchyma present, and rate of growth in length of epicotyl. S.D. represents significant difference for volume of statenchyma.

The statoliths, however, are well developed in all these species, but are limited to a single layer of cells surrounding the vascular bundles in the growing parts. *Amaryllis*, *Gladiolus*, *Iris*, and *Phoenix* on the other hand, have a quantity of starch present. It is, however, the embedded starch which is most variable among these species.

*Phoenix dactylifera*, the date, is worthy of note. Seedlings of this species are only slightly sensitive to gravity, the normal P.T. never being less than 24 hours. This may be due to (i) extremely slow growth, (ii) the compact nature of the seedling and the large amount of sclerenchyma present, or (iii) the check to curvature of the drag of the heavy 'stone'.

An attempt was made to eliminate the first factor by placing seedlings in an incubator at 30° C. in order to increase the rate of growth. Of two batches of seedlings at the same stage, placed horizontally, the batch at 20° C. showed no curvature until 48 hours after the commencement of stimulation, while those at 30° C. curved after only 24 hours stimulation.

If an attempt be made to bend the seedling between the fingers, considerable resistance is felt. The construction of the seedling is such that the greatest possible mechanical strength is obtained, the leaves are tightly

folded within one another and the large amount of sclerenchyma present is illustrated in Fig. 13. No attempt at reducing the amount of sclerenchyma by altering the external conditions was successful.

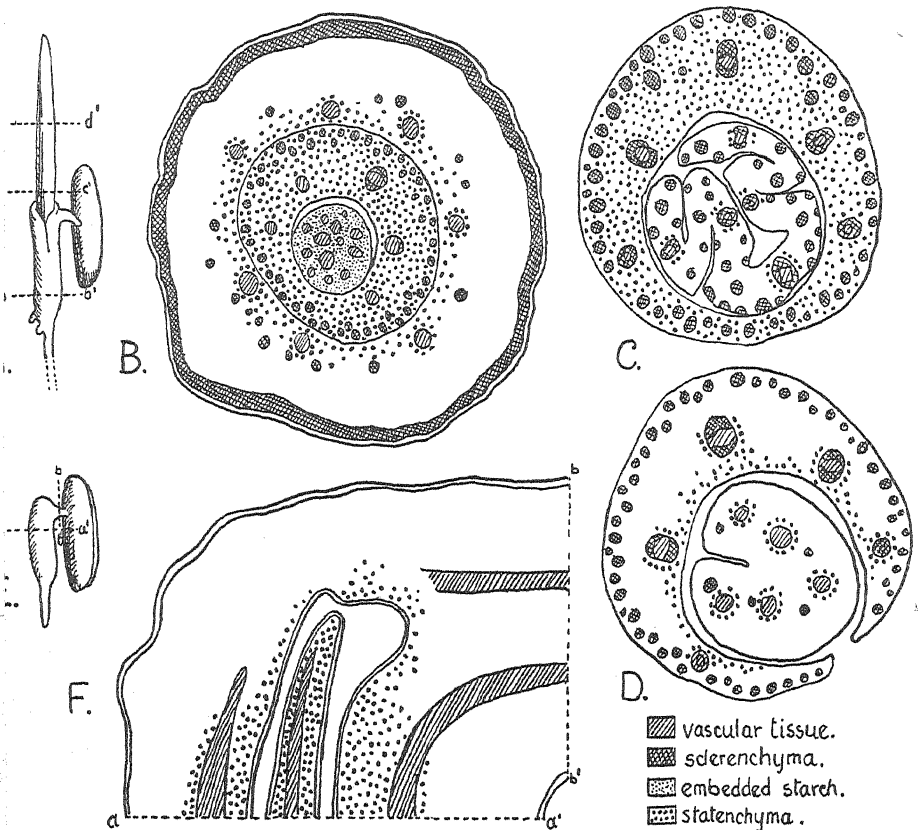


FIG. 13. A. Seedling of *Phoenix dactylifera*.  $\times \frac{3}{2}$ . B, C, D. Transverse sections through planes *b-b'*, *c-c'*, *d-d'* of A respectively.  $\times 16$ . E. Younger seedling.  $\times \frac{3}{2}$ . F. Longitudinal section of E between planes *a-a'* and *b-b'*.  $\times 16$ .

Some experiments were performed to test the influence of the third factor mentioned above. The cotyledon was severed at the point where it emerged from the testa, and by this means the P.T. was reduced by about 4 hours.

By these means it was found that the cotyledon is fairly sensitive to gravity in young seedlings, but that this sensitivity, or more probably the power of response, is lost as the sclerenchyma is formed. The foliage leaves are also sensitive when young, and the young first foliage leaf curves slightly inside the cotyledonary sheath when stimulated, although further curvature is prevented by the rigidity of the cotyledon. It is of interest, too, that the leaves curve at the tips and not at the base as is usual among monocotyledons.

The statolith apparatus, however, is particularly well developed in *Phoenix*. In the young seedling, before the first foliage leaf breaks through the cotyledon, embedded starch is present in the part of the cotyledon still enclosed in the testa, while the whole of the ground tissue of the young leaf and that part of the cotyledon immediately sheathing the leaf contain numerous statoliths (Fig. 13, E, F). Statoliths are also found in the cells of the columella of the radicle. The statocytes are large and often more than half full of large statoliths. When the first foliage leaf emerges from the cotyledon there are very few statocytes left in the cotyledon, and those present are confined to the base, while the whole of the ground tissue of the hypocotyl and the basal part of the leaf consists of statenchyma. Further up the leaf, the statenchyma is limited to the inner upper side, and at the extreme apex it is reduced to a single layer surrounding the vascular bundles. In the young second foliage leaf, statoliths are found in the bundle sheath and embedded starch is present in the basal part (Fig. 13, A-D). As the seedling develops there is less statenchyma in the cotyledon and the first foliage leaf.

There is an apparent difficulty here, in accounting for the presence of such a large quantity of statenchyma in a seedling which is so feebly geotropic as *Phoenix*. It seems probable, however, that this large amount of statenchyma is related to increased mechanical resistance to bending. It might be suggested that, after the initial orientation, the seedling has, on account of its own rigidity, no longer any need of geotropic curvature and that it has lost the power of response to the stimulus perceived by its own statolith apparatus. This is unlikely to be the case, since the seedling has been shown to be definitely geotropic.

##### 5. CONIFERS.

Twelve species of conifers were examined, all of which were members of Pinaceae or Cupressineae, the species studied being:—*Larix europaea*, *Picea pungens glauca*, *Pinus strobus*, *P. laricio*, *Pseudotsuga Douglasii*, *Abies Nordmanniana*, *Tsuga canadensis*, *Cupressus Lawsoniana*, *C. arizonica*, *C. macrocarpa*, *Cryptomeria japonica*, and *Thuja plicata*. Species of *Araucaria* and *Taxus* unfortunately failed to germinate.

Values for P.T. and L.T. have been worked out for the hypocotyls of *Larix*, *Picea*, and *Cupressus arizonica* (Table XIII) and show a general similarity in shape to those for hypocotyls of epigeal dicotyledons, but with certain differences which can be correlated with slower rate of growth.

A fall in P.T. to a minimum is seen, as in all seedling organs examined, but this minimum is never less than 25 min., and the subsequent rise in P.T. is very rapid, graviperception ceasing with cessation of growth in length of hypocotyl. The graviscripts for L.T. for the three species studied

show a similar shape to those for P.T., the minimum values for L.T. being at the same stage of development as those for P.T. The L.T. is always much greater for hypocotyls of coniferous seedlings than for those of dicotyledonous seedlings. The lowest L.T. obtained for conifer hypocotyls is 75 min. (*Larix europaea*), while *Abies Normanniana* has a minimum of four hours. In dicotyledons, the range of minimum L.T. values is from 30 to 70 min. In this respect conifer seedlings are more comparable with fronds of *Asplenium bulbiferum*, for which values of  $4\frac{1}{2}$ –7 hours for L.T. are recorded by Waight (32). Experiments performed on the other species, while insufficient to enable accurate graviscrits to be plotted, sufficed to show that all twelve species examined are very uniform in their response to gravity. There is an early positive curvature seen in the 'hook' of the young hypocotyl which has been discussed by Sperlich (28) and which is similar to that noticed in hypocotyls of dicotyledons.

TABLE XIII.

Length of hypocotyl in cm.	<i>Larix europaea</i> .		<i>Picea pungens glauca</i> .		<i>Cupressus arizonica</i> .	
	P.T. in min.	L.T. in min.	P.T. in min.	L.T. in min.	P.T. in min.	L.T. in min.
0-1.5	60	110	45	90	—	—
1.5-2.5	25	75	30	80	60	129
2.5-3.5	40	105	30	105	40	90
3.5-4.5	40+	105+	60	120	65	120

TABLE XIV.

Length of hypocotyl in cm.	<i>Larix europaea</i> .		<i>Picea pungens glauca</i> .	
	Relative sensitivity to gravity.	Volume of statenchyma in hypocotyl in cubic mm.	Relative sensitivity to gravity.	Volume of statenchyma in hypocotyl in cubic mm.
0-1.5	16	(1.0) <sup>1</sup> 0.382	22	(1.1) <sup>1</sup> 0.306
1.5-2.5	40	{ (1.8) 0.681	33	{ (1.7) 0.719
2.5-3.5	25	{ (2.33) 1.076	33	{ (2.13) 1.175
3.5-4.5	—	(3.03) 0.33	16	(3.0) 0.815

The distribution of starch in the hypocotyl is somewhat similar to that described above for the hypocotyl of a dicotyledon. In the young hypocotyl, before the 'hook' is straightened out, there is a quantity of fairly large grained, embedded starch in the cortex. Below the 'hook' region, statoliths are present in a loosely defined area consisting of from two to four layers of cells surrounding the vascular strands, with the exception of

<sup>1</sup> Figures in brackets represent actual mean lengths of hypocotyls in the samples used.

*Cupressus Lawsoniana* and *Thuja plicata* which, like the dicotyledons, have only a single layer of statocytes.

In older seedlings (Fig. 14), the embedded starch decreases in amount

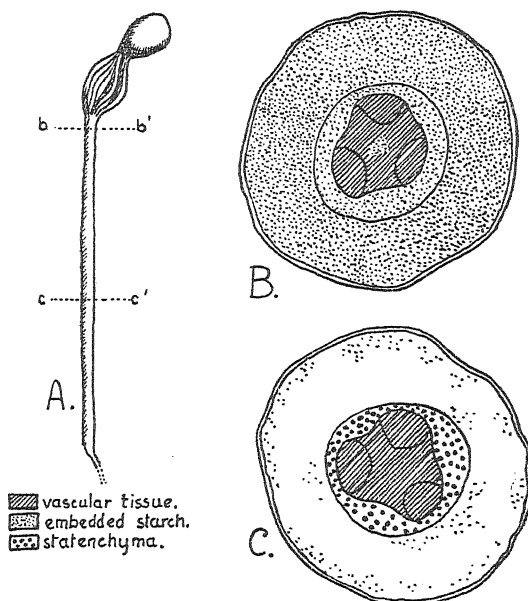


FIG. 14. A. Seedling of *Picea pungens glauca*.  $\times 23$ . B, C. Transverse sections through planes *b-b'* and *c-c'* of A respectively.  $\times 37$ .

and the statoliths in the lower part of the hypocotyl disintegrate. Later, the statoliths disappear entirely from the hypocotyl with the loss of geotropic sensitivity, but can be seen for a short time in the bundle sheath of the cotyledons and appear in the epicotyl as soon as it begins to elongate.

In the conifers studied, the average size of the statoliths does not differ greatly from that of the embedded starch grains (Table XV). The statenchyma is thus not marked off so sharply from the rest of the cortex as in dicotyledons, since not only are statoliths and embedded grains of similar size, but there is a transitional zone (surrounding the statenchyma), in the cells of which the grains are not all free to fall, and this suggests a lower degree of specialization of the statolith apparatus. It is of interest to compare this with fronds of *Asplenium bulbiferum* (18) where all the cortical cells are statocytes and there is no specialisation of a few cells for graviperception.

The volume of statenchyma at different stages in the growth of the hypocotyls of *Larix* and *Picea* was calculated, and a close correlation with geotropic sensitivity can be traced (Table XIV and Fig. 15).

TABLE XV.

Species.	Minimum P.T. in min.	Average diameter of statocyte in microns.	Average diameter of statolith in microns.	Average diameter of embedded starch grain in microns.
<i>Larix europaea</i> . . . .	25	25	4.5	5
<i>Picea pungens glauca</i> . .	30	22	5	5.5
<i>Cupressus arizonica</i> . .	40	19	4	4

## 6. DISCUSSION OF RESULTS.

Waight (32) has pointed out the necessity in geotropic work on fern fronds, of using stage, rather than length, as a criterion of development. On the other hand, with seedlings it is necessary to use length rather than stage, since it is impossible to define a close enough series of stages to bring out the essential changes in sensitivity. Moreover, since the growth rate of seedling organs is less variable than that of fern fronds, the objections, to the use of length with the latter, do not apply to any great extent with seedlings.

The existence of a positive geotropic curvature at the tips of the hypocotyls of certain dicotyledonous and coniferous seedlings, and of the young epicotyls of certain hypogeal species of dicotyledons, is a point of considerable interest from the point of view of the morphology of the statolith apparatus. Tupper-Carey (31), working with hypocotyls of *Helianthus annuus*, accounts for the two opposite geotropic curvatures in the hypocotyl by correlating the first positive curvature of the 'hook' region induced by nutation, with a state of active division in the vacuolating cells near the apex and by correlating the more typical negative curvature with a region of cell extension only, and suggests that positive curvature of the root is due to the greater extent of the region of cell division. It may be suggested, however, that the positive and negative geotropic curvatures can also be correlated with the position of the statoliths. In the young 'hook' region of the hypocotyl the starch has not yet become free to fall, while in the older part, where the negative curvature takes place, typical statoliths are found. Later, when the 'zone of efficiency' extends to the apex of the hypocotyl, the positive curvature disappears. In the root, the region where statoliths are found is confined to the root caps, and there are no statoliths present in the region of positive curvature. It was suggested by Tupper-Carey (31) that a slight negative curvature may actually take place in the extreme root tip, i.e. in the statolith area. Thus it seems at least possible that a direct stimulus produces a negative response, and an indirect or conducted stimulus produces a positive response. The possibility that the difference between direct and indirect stimuli might account for the difference in response in root and stem has



been pointed out by Bose (2), but without direct reference to the statolith apparatus.

From a consideration of the dicotyledonous species used, it appears that there is no real difference between those having hypogeal and those

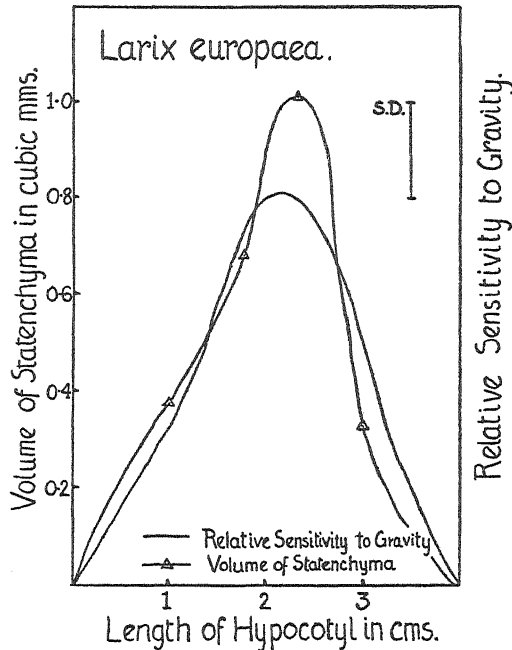


FIG. 15. *Larix europaea*. Graph to show relation between sensitivity to gravity and volume of statenchyma in the hypocotyl. S.D. represents significant difference for volume of statenchyma.

having epigeal germination, from the point of view of the geotropism and statolith apparatus of the seedling. The short hypocotyl of the hypogeal forms undergoes similar changes in sensitivity and the same development of the statolith apparatus as that of the epigeal forms in a much shorter time. Although critical experiments were discontinued after the elongation of the hypocotyl, in those dicotyledons having epigeal germination, comparative experiments show that the geotropism and the morphology of the statolith apparatus in the epicotyl is similar to that recorded above for *Lathyrus odoratus*.

Seedling organs of monocotyledons show, on the whole, a shallower range of values and a more gradual change in both P.T. and L.T. than do the more rapidly elongating hypocotyl and epicotyl of the dicotyledons, and are in general less sensitive (as shown by high minimum P.T.) than the latter. The epicotyl of *Asparagus officinalis* is a notable exception, and it is of interest that this species approaches such a hypogeal dicotyledon as *L. odoratus*, both in its mode of germination and in the geotropism of the

seedling. In the other monocotyledonous species studied, it may be noted that the cotyledon is physiologically comparable to the hypocotyl of an epigeal dicotyledon. The greater variety shown by the monocotyledonous seedlings both in perception and response to gravity and in the morphology of the statolith apparatus is a direct consequence of the greater variety of methods of germination shown by these plants than by the dicotyledons.

The geotropism of the hypocotyl in coniferous seedlings is essentially similar to that of the dicotyledonous hypocotyl, but the higher minimum values and the steeper rise in both P.T. and L.T. are probably the result of slower growth. Coniferous seedlings are very uniform both in their geotropism and in the morphology of their statolith apparatus, which is what would be expected in so natural a group of plants.

Thus it can be stated as a general rule that in all seedling organs of limited growth (e.g. the hypocotyl or a single node of the epicotyl) the geotropic P.T. and L.T. first fall to a minimum value and then rise, sensitivity being lost with cessation of growth in length, while in all seedling organs of unlimited growth (e.g. the epicotyl), P.T. and L.T. first fall to a minimum value and then rise to some constant value at which they remain as the young plant develops. The graviscritps for P.T. and L.T. of any seedling organ are more or less parallel curves, the minimum values occurring at the same stage in development, which is in accordance with the results of Waight (32) and Prankerd (19, 20) for fern fronds.

From the study of the statolith apparatus in seedlings there appears to be some correlation between size and resistance to bending in plant organs and the amount of statenchyma present. The example of *Phoenix dactylifera* is cited above. Among conifers, the very slender hypocotyls of *Cupressus Lawsoniana* and *Thuja plicata* have only a single layer of statocytes, while the more massive species have more than one row of statocytes, a tendency which culminates in the presence of four or more rows in *Abies Nordmanniana*, which has the most inflexible hypocotyl of any conifer studied. Among dicotyledons, a double layer of statenchyma, with few exceptions, e.g. *Vicia Faba*, is only found in the more massive seedlings of arboreal species, e.g. *Aesculus hippocastanum*, *Quercus* sp., *Fagus sylvatica*, *Crataegus Oxyacantha*, and *Citrus paradisi*, while slender seedlings of arboreal dicotyledons, e.g. *Salix* sp., *Pyrus malus*, *Laburnum vulgare*, *Carpinus Betulus*, like the herbaceous species, have only a single layer of statenchyma.

It is of significance that, in the investigation of the geotropism and statolith apparatus of seedlings recorded above, no case has arisen which is not in accordance with the statolith theory of geotropism. In all cases of bilateral symmetry in geotropism which have been examined, the morphology of the statolith apparatus would seem to provide a very complete explanation, and the fact that this phenomenon, hitherto inexplicable, can

be explained in this way is of importance, since it suggests an entirely new line of evidence in support of the statolith theory.

The small amount of variation seen in the volume of statenchyma present, at any particular stage, in any of the seedling organs examined quantitatively is of significance when compared with the great variability in the presence and amount of embedded starch, which is in accordance with the qualitative results obtained for branches of trees (22).

In all seedlings examined quantitatively the very close correlation between the volume of statenchyma and the degree of sensitivity to gravity is of special significance. For this correlation between statoliths and geotropism, both in time and space, together with the fact that the morphology of the statolith apparatus can provide an adequate explanation of the phenomenon of bilateral symmetry in the geotropism of seedlings, seems to give considerable support for the statolith theory of geotropism. Janse's (11) claim that the statocyte can act as a sensory organ for the perception of *any* type of stimulus may, in the present state of our knowledge, be rather extravagant, yet it seems fairly conclusive that it can and does act as the perceptive organ for gravitational stimuli.

#### SUMMARY.

1. Values for presentation and latent times during development have been worked out for seedling organs of 15 species, chosen from among Dicotyledons, Monocotyledons, and Conifers. For organs of limited growth, e.g. the hypocotyl, the values for presentation time and latent time first fall to a minimum and then rise, sensitivity ceasing with growth in length. For aerial organs of theoretically unlimited growth, e.g. the epicotyl, the values of presentation time and latent time show a fall to a minimum value and a subsequent slight rise to some constant value. Comparative work on other species confirms these results.

2. Seedlings of Monocotyledons and Conifers are, with few exceptions, less sensitive to gravity than those of Dicotyledons.

3. Values for presentation time and latent time for primary roots fall to a minimum value, at which they remain.

4. The statolith apparatus has been examined in about 80 species. Statoliths appear first in the root cap of the radicle. In the hypocotyl or epicotyl of seedlings of Dicotyledons the statoliths are usually found only in the endodermis. In seedlings of a few arboreal Dicotyledons and of some Monocotyledons and Conifers a statolith-containing tissue or statenchyma is found.

5. An attempt has been made to determine quantitatively the amount of statenchyma present at different stages of development of seedlings of each of the three groups, and the results show a very close correlation with sensitivity to gravity.

6. The morphology of the statolith apparatus provides an explanation of the phenomenon of bilateral symmetry in the geotropism of certain seedlings.

7. The results obtained are in accordance with the statolith theory of geotropism.

My sincere thanks are due to Professor J. R. Matthews for the encouraging interest which he has taken in this investigation, and to Dr. T. L. Pranker for her kind advice and criticism.

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# The Distribution of Water in the Shoots of certain Herbaceous Plants.<sup>1</sup>

BY

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AND

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With four Figures in the Text.

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<sup>1</sup> Owing to the death of Professor R. H. Yapp this paper has been prepared for press by the second author alone.

## 1. INTRODUCTION.

IN a previous paper by one of us (4) it was shown that the upper leaves on an erect flowering shoot of *Spiraea Ulmaria* differ markedly in structure from the lower leaves on the same shoot. The upper leaves, for instance, are thinner and more hairy: their cells are smaller, and in consequence the stomata and other elements are more numerous per unit of area or volume than are those of the lower leaves.<sup>1</sup> It was suggested (*loc. cit.*, § 11, p. 842, &c.) that certain of these structural differences might be due to differences of turgor during development. The evidence available at the time was mainly indirect, and in any case the stage of leaf-development at which the mature structure was determined was undecided. We have therefore attempted to investigate the question further, by determining the water-content of leaves in various stages of development.

This paper records the results of our investigations of the water-content of the apical bud and the various leaves of the shoot of selected herbaceous plants. Further, an attempt is made to interpret the water-content in terms of turgor, and to ascertain the condition of turgor during the developmental stages of the leaf.

The water-content of different plant organs has been determined by many investigators, often in connexion with more general chemical analyses. The results of such determinations prior to 1882 were summarized by Ebermayer (3). He concluded that younger plants contain in general more water than older. The actual amounts of water recorded (in percentages of fresh weight) varied from 38 per cent., the minimum in freshly felled trunks of hard wood trees, to 98 per cent. in the case of certain algae. The leaves of trees and shrubs (in August) varied from 54 to 70 per cent., of herbaceous plants from 60 to 80 per cent., while succulents such as *Cacti* and *Sedum* contained 90 to 95 per cent.

The most important earlier paper from our point of view is one by von Hoehnel (4), the section of which dealing with the water-content of leaves appears to have been overlooked by many later investigators.<sup>2</sup> Von Hoehnel wished to study the variations in water-content of the leaf during the course of its life. As this could not be determined directly, he employed consecutive leaves of one and the same shoot, as representing successive ages and therefore stages of development. Von Hoehnel fully recognized that this indirect method introduced a probable source of error:

<sup>1</sup> Zalenski (1904), in an admirable paper, has shown conclusively that the structure of the leaves of ordinary herbaceous plants is a function of the position of the leaf on the stem. This paper, being in Russian, has not attracted the attention it deserved.

<sup>2</sup> We ourselves only saw this paper after most of our experimental results had been obtained.



but he was unaware that mature leaves at different levels on the same shoot often differ markedly in structure (Zalenski, Yapp), and these structural differences involve differences in water-content. Although the assumption on which von Hoehnel's work was based was not fully justified, this only affected certain of his conclusions, and did not vitiate the valuable results actually obtained. He distinguished three main types of plants. (1) *Herbs and deciduous trees*, in which the water-content usually decreases from the apical bud to a minimum in leaves of a certain age, then increases to a second and higher maximum in older but still vigorous leaves. (2) *Urticaceae*: the water-content continually decreases from the youngest to the oldest leaves. (3) *Evergreen plants* also show, with various modifications, a gradual decrease with age.

Sluyter (12), with a similar object in view, but using different methods, repeated von Hoehnel's work. On the whole his results confirm those of von Hoehnel as far as groups (2) and (3) are concerned. But he divides group (1) into: (a) *herbs*, in which the minimum occurs in the youngest leaves, the water-content steadily increasing with age, and (b) *deciduous trees and shrubs*, which show a more or less regular decrease with age, except for a peculiar slight rise at some point during development, and a final rise when the leaf is yellowing.<sup>1</sup>

More recently Krasnoselsky-Maximov (8), also Maximov and Krasnoselsky-Maximov (11), have found in various herbs a gradual decrease in the percentage of water in leaves taken from successively higher levels on the stem. But so far von Hoehnel alone has recorded a second maximum in the apical region of herbaceous shoots. There is less evidence regarding other classes of plants, but many appear to show a more or less reversed curve of water-content, the lower leaves containing less water than the upper.

Under normal conditions, then, different leaves on one and the same shoot show considerable differences in water-content, depending on the relative positions of the leaves on the shoot. It will be seen later that this is the case even when the whole plant is in as complete a stage of turgor as can be produced experimentally. In addition to the normal and often wide range due to position the actual water-content of a leaf may exhibit considerable fluctuations according to environmental conditions. In general, conditions favouring evaporation, e. g. high temperature and high humidity deficit, decrease the water-content. Thus Livingstone and Brown (9),<sup>2</sup>

<sup>1</sup> The differences between von Hoehnel's and Sluyter's results as regards deciduous woody plants may be partly due to differences in the methods employed. Von Hoehnel collected all the leaves of a shoot simultaneously, while Sluyter usually took the oldest leaves only, at more or less equal intervals throughout the summer. The latter method, while giving the water-content of leaves of successive ages from a similar position on the shoot, introduces variations associated with the march of the seasons.

<sup>2</sup> Livingstone and Brown appear to have been unaware of the wide range in water-content due

Krasnoselsky-Maximov (8), Knight (5), and others have found more or less marked diurnal variations in the water-content of leaves. There is some evidence that seasonal variations may also occur,<sup>1</sup> but here it is difficult to disentangle the effects of changes of atmospheric and other soil conditions, due to the march of the seasons, from those due to progressive structural changes occurring in the leaves themselves.

So far as herbaceous plants are concerned, all the authors mentioned above have confined their attention to the water-content of leaves.

The experiments recorded in these papers were begun in Belfast in 1919, and continued in the Edgbaston Botanic Gardens, Birmingham, during 1921 to 1924. The earliest experiments were made with *Spiraea Ulmaria*, L. (= *Filipendula Ulmaria*, Max.), but subsequently, on account of the great numbers of plants required, the annuals *Helianthus annuus*, L., and *Vicia Faba*, L., and in some cases other species, were employed. Especial attention has been paid to the younger, distal portion of the shoot, with leaves in various stages of development; but as the work progressed other parts, including the stem, were also investigated.

#### METHODS.

The plants were grown in open soil or, for certain purposes, in large pots. The material was cut from growing plants and *immediately* transferred to numbered chemical weighing-bottles of sizes suitable for the amounts of material concerned. The stoppered bottles were transferred in dark boxes to the laboratory, weighed, and heated for four to eight hours, according to the amount of material, in a Hearson electric drying-oven regulated to about 97° C. The bottles were then heated for 20 minutes in a second oven at about 105° C., cooled in a desiccator and again weighed. The process was repeated, with 20 minutes' heating (at 105° C.) each time, till weight was constant. As dried plant material is highly hygroscopic, the stoppers were only removed while in the drying-oven, the bottles being loosely stoppered in the desiccator, and lightly during weighing.

The drying process may possibly cause loss of other volatile substances in addition to water. To minimize the danger of this, drying *in vacuo* at a lower temperature (about 40° C.) was tried: but complete desiccation was

to position, for they make no reference to the positions on the stems from which leaves were collected. But as in this case the authors (p. 319) used 'a large number of similar leaves' (presumably discarding very old as well as obviously immature leaves), the danger of serious error in the results obtained was probably eliminated. Lloyd (10, p. 11) in a somewhat similar investigation took the precaution of selecting leaves growing at the same distance from the ground.

<sup>1</sup> Cf. Ebermayer (3, p. 6). Sluyter's (12) tabulated results suggest seasonal variations. Clark (2) found in the leaves of certain trees a gradual decrease in water-content during the summer (with an August minimum) and a rise in autumn; but this author's somewhat crude experimental methods introduce uncertainty as to the reliability of her results.

difficult and the method proved too slow when large numbers of bottles were involved.<sup>1</sup> For our purposes, the relative rather than the absolute water-content is required, and it seems improbable that the loss of other volatile substances will seriously affect the results, even though the proportion of such substances should vary somewhat in different parts of the plant.

The results recorded in this paper are usually calculated as percentages of fresh weight, and not, as has been advocated by some recent authors, as percentages of dry weight.<sup>2</sup>

In some cases single leaves were used. Usually, however, especially in experiments involving the use of minute, partly developed leaves, from two to six plants (generally four) of the same age, height, and number of leaves, growing under similar conditions, were selected for each experiment. Leaves of the same stage and age were included in the same bottle, and an *average* water-content thus determined. By this method of averaging more homogeneous and reliable results were probably secured, especially in the younger stages of leaves which involved the use of very small quantities of material. Internodes were taken singly.

## 2. THE WATER-CONTENT OF LEAVES.

(a) *A few typical curves showing the water-content of successive leaves* of vigorous growing shoots of *Helianthus* and *Vicia* are given in Fig. 1. All the leaves on a shoot were gathered (in order from below upwards) and bottled as rapidly as possible, and the water-content determined. The water-content—in percentages of fresh weight—is plotted as ordinates against the successive leaves of the shoot, which differ in age and in position on the stem, as abscissae. These curves show: a maximal percentage of water in the lowest leaf taken; a gradual decrease in the young

<sup>1</sup> Koketsu (6 and 7) has suggested a new method of determining water-content, based on the total volume of pulverized tissue obtained from the material investigated. Koketsu claims that his 'Pulvermethode' is under certain conditions more suitable than the usual percentage methods; we have not tried this method, but the way in which the volume is ascertained would seem to introduce a possible source of error.

<sup>2</sup> Krasnoselsky-Maximov (8), for example, maintains that variations in solid substance are (for this purpose) negligible, and therefore that calculations based on dry weight permit one to follow the fluctuations in water-content more closely. But the absolute amount of solids may vary appreciably, while variations in the water-content of leaves, at all events in climates such as that of the British Isles, may be smaller than is sometimes assumed (cf. discussions in Knight (5) and Chibnall (1), also the apparently simultaneous loss of water and solid substances during prolonged wilting recorded by Maximov, Krasnoselsky-Maximov (11)). Normally the apparent water-content would of course tend to be decreased during the day by the accumulation of assimilates, and progressively increased during darkness by reasons of translocation and respiration. Since both water content and dry substance are variable quantities, the method of calculating the results is immaterial. The older fresh weight method is the simpler, as it avoids the high figures obtained when water-content is calculated in terms of dry weight.

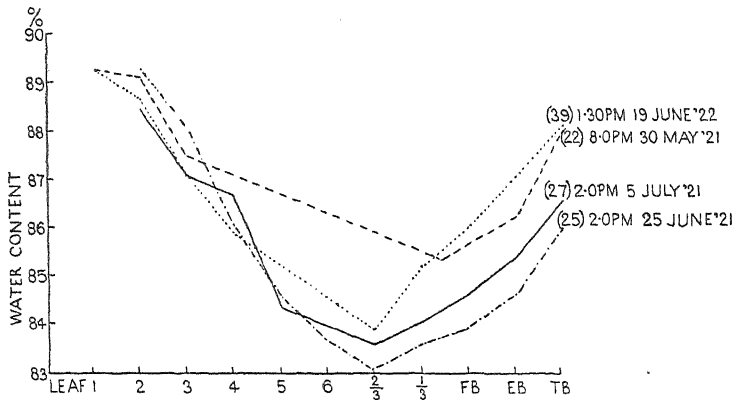


FIG. 1 a.

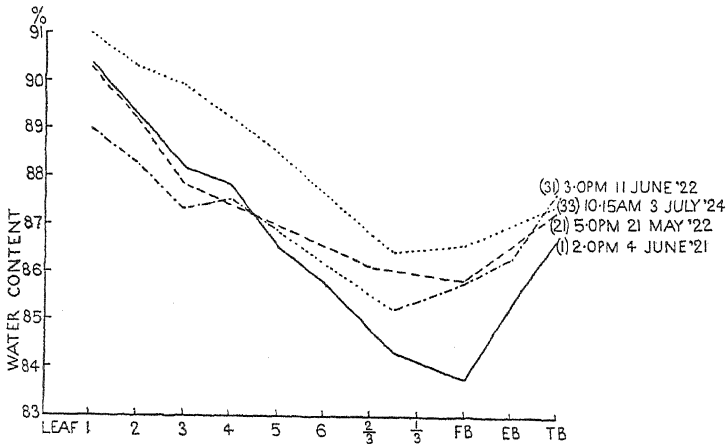


FIG. 1 b.

FIGS. 1. A and B. Typical water-content curves for successive leaves on the same shoot. 1 A, *Helianthus annuus*, 1 B, *Vicia Faba*. Leaf No. 1 is the lowest and oldest foliage leaf on the stem; leaf No. 2 the second, and so on.  $2/3$  and  $1/3$  indicate unfolded and flattened, but not yet fully expanded leaves. The fractions represent roughly the sizes of these immature leaves as compared with their estimated sizes at maturity. F.B., younger leaves (often still folded) which have just freed themselves from the terminal bud, and are therefore more or less exposed on all sides to the atmosphere. E.B., leaves enclosing the terminal bud: their lower surfaces are more or less exposed, the upper being still in contact with the bud. T.B., the terminal bud, including the stem apex and the minute leaves and leaf rudiments: for the most part these were not directly in contact with the atmosphere. The times of the experiments cited have all been converted to winter (Greenwich mean) time. The varied experiments are denoted by varied dotted or dashed lines. In cases where the plants were not tall, and few stages of development were represented, the points of the basal and apical rosette leaves are allowed to coincide with those of older plants, the gap between being joined by a straight dotted line. The numerals cited are the record book numbers which have been retained for the convenience of the authors.

leaf at a certain stage of development, and, finally, a steady increase in still younger leaves to a secondary maximum in the terminal bud.<sup>1</sup>

Fig. 2 contains three composite curves giving the average results of many comparable experiments. H is from plants of *Helianthus* growing

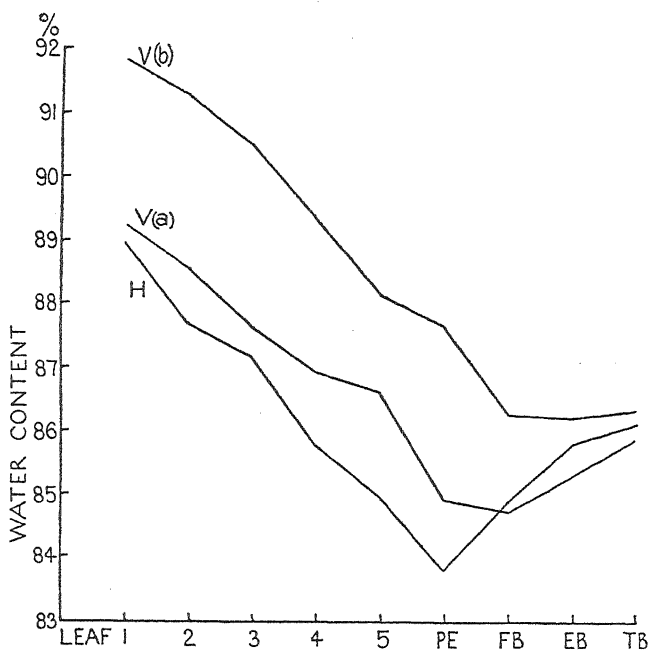


FIG. 2. Composite curves giving the average water-content determinations for many comparable plants. H and v(a) are water-content curves of plants of *Helianthus annuus* and *Vicia Faba* grown in the open soil in exposed situations. v(b) is the curve of plants of *Vicia Faba* grown either in shade or under humid conditions. The lettering of the leaves is the same as in Fig. 1.

in open soil with fully exposed shoots. v(a) is a curve from similarly exposed plants of *Vicia*. v(b) (also *Vicia*) is constructed from plants either grown permanently in decreased light, or else placed temporarily in humid air—both sets of conditions being such as favour increased water-content of the leaves.

The general form of curve shown in Figs. 1 and 2 is normally found in all healthy and still growing shoots before the lower leaves begin to wither and die. Such curves indicate the relation between the position of leaves on the stem and their water-content. The causes of the differences due to position will be discussed later.

<sup>1</sup> Von Hoehnel (4) seems to be the only previous investigator to note this secondary rise in the apical region of the shoot. He selected, however, very few younger stages (mainly the terminal bud), and apparently omitted the precaution of bottling his material before weighing.

(b) *The normal positions of the maximal and minimal water-content.* So far as foliage leaves are concerned, the *absolute maximum* almost invariably occurs in both *Helianthus* and *Vicia* in the leaf or leaves of the lowest node, provided that these lowest leaves are still in full vigour and that the plants are not in a wilting condition (see Figs. 1 and 2). The fresh cotyledons of *Helianthus*, however, contain an even higher percentage of water than the lowest foliage leaves. E.g.—Exp. H 44, cotyledons—93.88 per cent.; first pair of foliage leaves—91.41 per cent.; second—89.94 per cent., and so on. The only exceptions to this rule actually observed were (i) in wilting plants, when the upper leaves had probably abstracted water from the lower (see p. 176). (ii) In relatively old, tall plants, in which the lowest leaves had begun to fade, the maximum occurred in a still vigorous leaf somewhat higher on the stem. This confirms von Hoehnel's assertion that the water-content tends to fall when the leaf has passed its period of greatest activity. (iii) In four experiments (out of thirty-seven) with *Vicia*, leaf 2 had a slightly higher water-content than leaf 1: in these cases the plants were fairly tall and leaf 1, though apparently vigorous, may have been declining in activity.

The position of the *absolute minimum* is more variable than that of the maximum. In fairly young, vigorous plants of *Helianthus*, the minimum occurs in the great majority of cases in partly developed leaves which have expanded to (roughly) about one-third to two-thirds of their estimated ultimate size (Figs. 1 and 2). In a few cases the minimum was found in still younger leaves. E.g., out of fifty-nine experiments, five showed the minimum leaves just freed from the terminal bud (FB), one in leaves enclosing bud (EB), and five in the terminal bud. In four of the latter, however, the plants were very small and few developmental stages could be distinguished. In old plants of *Helianthus*, in which the apical leaves are more or less mature, the minimum is often in the highest leaf or leaves: this suggests that with maturity the position of the minimum shifts to the youngest leaves surrounding the apex of the stem.<sup>1</sup>

Although in *Vicia* in a few instances the minimum occurred in leaves half to two-thirds developed, in vigorous plants it is usually somewhat nearer the apex than in *Helianthus* (Figs. 1 and 2), the commonest positions being in leaves just freed from bud (FB), but with lamina not yet fully unfolded, and (less frequently) in leaves enclosing bud (EB). Occasionally the minimum was found in the terminal bud itself. It will be seen later that under certain conditions the position of the minimum may temporarily shift slightly either in an apical or in a basal direction.

<sup>1</sup> Throughout the text the following abbreviations have been used:

PE = 'partially expanded'.

FB = 'leaves just freed from apical bud'.

EB = 'leaves enclosing bud'.

TB = 'terminal bud'.

(c) *Averages and ranges in water-content of leaves of different ages and levels on the stem.* Table I (*Helianthus*) gives the average percentage water-content, the absolute maxima and minima, and the extreme ranges found in leaves in different positions on the stem. The figures have been obtained by combining the results of all experiments with healthy, growing plants of various sizes, rooted in soil in the open, and with shoots exposed to ordinary atmospheric conditions (see also Fig. 2). The numbers of experiments on which the various determinations are based are given at the bottom of the table. Letters PE, FB, &c., as in Figs. 1 and 2.

TABLE I. (*Helianthus*).

Tier of leaf.	1 (lowest).	2.	3.	4.	5.	PE.	FB.	EB.	TB.
Average water-content	88.98	87.69	87.19	85.78	84.97	83.81	84.88	85.80	86.14
Absolute maxima	90.25	89.41	88.27	86.73	85.52	86.84	86.34	87.17	88.16
Absolute minima	87.27	84.86	85.57	84.27	84.42	79.65	82.05	83.66	84.11
Extreme range	2.98	4.55	2.70	2.46	1.10	7.19	4.29	3.51	4.05
No. of experiments	11	16	8	7	4	43	13	15	37

This table shows that on the whole the extreme ranges of water-content are greater in the younger leaves of the apical region than in the older basal leaves. This, however, must not be regarded as absolute. In the case of extreme drought, as will be seen later, the lower leaves lose water more rapidly than upper leaves. If such cases were included, the ranges of the lower leaves would be increased. It is further seen that the maximum range (7.19 per cent.) is found at the same stage of development (PE) as the average minimum water-content (83.81 per cent.). It will be seen later that this indicates that the greatest fluctuations of turgor occur, under normal conditions, in these partly developed leaves. Possibly the large number of experiments (e. g. forty-three in PE and thirty-seven in TB) may in part account for the wider range obtained in these regions as compared with certain others (e. g. only four in tier 5).

But that the number of plants employed does not do more than accentuate differences of range is seen from the following figures for *Vicia* (see also Table II), which combine, as in Table I, the results of experiments with exposed plants growing in open soil. The figures in parentheses give the numbers of experiments.

*Vicia.* Leaf 1 (lowest)—average water-content 89.21 per cent., extreme range 2.06 per cent. (No. of experiments 10).

Leaf 2. 88.56 per cent., range 2.01 per cent. (9).

„ 3. 87.63 „ „ 1.64 „ (10).

„ 4. 89.93 „ „ 2.51 „ (7).

„ 5. 86.63 „ „ 1.62 „ (6).

Leaf 6.	86.21	per cent.,	range 1.91	per cent.	(6).
„ 7.	86.03	„	„	1.17	„ (3).
„ 8.	85.89	„	„	0.98	„ (3).
„ 9.	85.54	„	„	1.63	„ (2).
PE.	84.93	„	„	2.99	„ (8).
FB.	84.73	„	„	4.49	„ (12).
EB.	85.30	„	„	2.31	„ (7).
TB.	85.88	„	„	3.35	„ (12).

The results with *Vicia* confirm those obtained from *Helianthus*, i.e. in general the extreme ranges are greater in the younger than in the older leaves, and the maximum range (4.49 per cent.) is found at the same stage of development (in this case in leaves just freed from the terminal bud) as the average minimal water-content (84.73 per cent.). Similar results were obtained in the case of humidity experiments with *Vicia*: these are recorded in Table II, p. 172.

(d) *Effect of the age and height of a plant on the average water-content of its leaves.* From the facts mentioned above it might be expected that, other things being equal, the average water-content of the leaves of a shoot would diminish as the age and height of a plant and the number of its leaves increase. This was almost invariably found to be the case. In *Helianthus*, for example, in nine experiments with seedlings 2–3 in. high, in open soil (June 1919 and 1922), the average number of stages distinguished was 3.5, and the average water-content 86.67 per cent. In six experiments with plants 7–9 in. high (May and June 1921 and 1922) with an average of six stages, the average water-content was 86.78 per cent. In six experiments with plants 16–32 in. high (June and July, 1921 and 1922), and an average of nine stages, the average water-content was 85.64 per cent. With still older plants, the lowest 10–15 leaves of which were dead (and were excluded), still lower percentages were obtained. E.g., plants 5–6 ft. high just beginning to flower (September 1921, 1922) had an average water-content of 84.5 per cent. Similar plants in an early stage of fruiting (October 9, 1922) yielded an average of 81.8 per cent., while plants with ripening fruits (October 24, 1922) gave only 77.7 per cent. But height and age alone do not determine the absolute average water-content. For instance, a well-grown plant (6 ft. 6 in.) in open soil, not yet flowering (August 1921), showed an average of 86.66 per cent., although several of the lowest leaves were omitted; this is practically the same as that of the seedlings mentioned above.

The experiments so far cited were performed in different months and years, and under a variety of weather conditions. More striking evidence of the effect of age and height is afforded by two series of experiments in June 1922 (*H* 37–42 and *V* 28–32). Rows of seeds of *Helianthus* and



*Vicia* were sown each week for about six weeks. Subsequently the water-content of the leaves of plants of different ages and heights was determined simultaneously, four similar plants being used for each experiment. In all cases the plants were vigorous and still growing, and the lowest leaves healthy. The averages obtained for the entire plants were as follows:

(1) *Helianthus*:

Height of plant . .	2"	3"	9"	12"	16"	21" to 24"
Average water-content of all leaves of shoot	87.70 %	87.32 %	86.77 %	86.86 %	85.88%	85.93 %

(2) *Vicia*:

Height of plant . .	8"	11"	17.5"	19"	26"
Average water-content	87.38 %	87.14 %	86.79 %	86.16 %	85.99 %

These two series show on the whole a gradual decrease in the average water-content with increasing height, the *Vicia* series being the more regular of the two.

### 3. FACTORS INFLUENCING THE WATER-CONTENT OF LEAVES.

The ratio of solid matter to water in cells, and therefore in tissues and organs, is subject both to influences temporarily affecting the water balance, and also to those which, by long continued action during the growth period, affect the permanent structure of the plant.

(a) *Diurnal fluctuations in water-content.* The existence of a daily cycle of changes in the water-content of leaves has been definitely established by the work of several authors. Livingston and Brown (9), in the desert climate of Tucson, Arizona, found a considerable reduction in leaf moisture during the hours of maximum transpiration, in plants growing in the open. In terms of fresh weight, the differences in water-content between day and night varied from 8 per cent. (in *Martynia louisiana* and *Sida angustifolia*) to 1 per cent. (in *Physalis angulata* on a cloudy day with low evaporation), the average for 13 species being 4.5 per cent.

Mme Krasnoselsky-Maximov (8), in the arid but less extreme climate of Tiflis, obtained similar results with both xerophytes and mesophytes. In the leaves of *Artemisia fasciculata* the greatest diurnal difference observed was 7.7 of the fresh weight. With other plants such differences as 5.4, 4.3, and 2.1 per cent were obtained.<sup>1</sup> The author further investigated the changes in the water-content of leaves at different levels on the stem. She found a greater diurnal range in lower than in upper

<sup>1</sup> N. A. Maximov and T. A. Krasnoselsky-Maximov, in a later paper (Journal of Ecology, 1924), cite a daily deficit in the sunflower of 28 per cent. of the general water-content in the early morning hours, 26 per cent. in the potato, 13 per cent. in *Impatiens parviflora*, 14 per cent. in *Tussilago Farfara*, and 15 per cent. in *Chelidonium majus*.

leaves. For example, in an experiment with *Zygophyllum Fabago*, leaves at five successive levels on the stem showed the following diurnal ranges, respectively, in *percentages of dry weight*. The lowest leaves taken, 154.8 per cent.; those of the node above, 118.1 per cent.; next higher node, 103.1 per cent.; two nodes higher, 100.5 per cent.; four nodes higher (the highest taken), 99.3 per cent. Such differences of range of water-content the author interprets as indicating a movement of water from the lower to the upper leaves, the former acting as water reservoirs on which the upper leaves can draw when confronted with an adverse water balance. The work of both Brown and Livingston and Krasnoselsky-Maximov make it clear that these diurnal changes in the water-content of foliage leaves are closely associated with evaporation conditions and the supply of water from the roots—in other words, with the water balance of the plant. Livingston and Brown (9), from results obtained in cloudy weather, predicted that a diurnal decrease in leaf moisture 'may fail to occur in regions of low evaporation when accompanied by relatively high rates of soil moisture supply'.

In the cooler and more humid climate of S.E. England, Knight (5) found appreciable, but much smaller, fluctuations. For example, with *Eupatorium adenophorum* in a greenhouse, the average decrease in water-content for 12 pairs of leaves, between 8.30 a.m. and 1 p.m. (G.M.T.), was 0.5 per cent., the maximal decrease being 1.3 per cent. Three pairs showed an increase instead of a decrease. Knight's method with *Eupatorium* was to determine the water-content of one leaf of each pair at the earlier, and that of the other leaf at the later hour.

The main results of some of our experiments, performed in duplicate, usually at about 1 p.m. and 7 p.m. (G.M.T.), with either *Helianthus* or *Vicia*, may be briefly recorded. Four, or in some cases two selected plants, of the same height and age, and with the same number of leaves, and grown under the same conditions, were used for each experiment. In 15 out of 19 experiments, the average water-content of the leaves of varying ages (considered as a whole) was greater by night than by day. The greatest difference found was 2.77 per cent. and the least 0.02 per cent., the average being 1.01 per cent. In the remaining four experiments, the water-content was slightly greater, on the average by 0.45 per cent., in the early afternoon than during the evening. In 17 of these experiments the plants were growing in open soil, in the other two in large flower pots. Thirteen of the experiments (including the four in which the maximum was at 2 p.m.) were performed during the drought of 1921, but even in showery weather a difference of 1.42 per cent. was recorded on one occasion (June 1919).

The diurnal range of water-content in leaves at different levels on the same stem was more irregular in our experiments than in those of

Krasnoselsky-Maximov. In a few cases the range was greatest in the lower leaves, e.g. Expts. *H* 9-11 (June, 1919); times of experiment 1.40 and 8 p.m. and 3.15 a.m. Small plants: 2.07 per cent. (second leaf, the lowest taken); 1.96 per cent.; 1.25 per cent. (PE); 0.64 per cent. (TB). More frequently, however, the ranges were either more or less irregular, or else the greater ranges occurred in the upper leaves. E.g., Expts. *H* 29-30 (July 1921). Potted plants: the respective ranges 0.11 per cent. (second leaf, the lowest taken); 1.80 per cent.; 1.75 per cent.; 1.85 per cent.; 2.47 per cent. (PE); 3.75 per cent. (FB); 3.16 per cent. (EB); 3.39 per cent. (TB). In the second leaf the maximum water-content was at 1 p.m., in all other leaves at 8 p.m.

The result of the various investigations on the diurnal fluctuations in foliar moisture show that in dry regions with high evaporation a marked water deficit usually occurs during the day-time. Livingston and Brown's prediction regarding regions of low evaporation is to some extent borne out by our experiments and those of Knight. In the relatively cool and humid summer climate of the British Isles the water deficit is much less than in drier climates. Yet even here there is, in most cases, a small but appreciable adverse water balance during the hotter hours of the day. If this deficit is sufficiently great, it would seem (to judge from the greater diurnal range sometimes found in the lower leaves) that the upper leaves may draw upon the water reserve of the lower leaves. Under the conditions of our experiments, however, such transference of water appears to occur less frequently than in Krasnoselsky-Maximov's experiments.

(b) *Experiments in still, humid air.* (See Table II, *Vicia*; Table III, *Helianthus*.) In the experiments just cited the water balance is affected mainly by the natural diurnal march of meteorological factors coupled, no doubt, in the more extreme cases, with a restricted water-supply. It seemed advisable to supplement these experiments by others in which normally grown plants were placed under artificially humid conditions.

Four pairs of experiments were carried out with *Vicia*. In the first three pairs (*V.* 19 to 24)<sup>1</sup> the plants selected were rooted in soil in the open and used when about 8 in. high. With regard to the first pair, *V.* 20 and *V.* 19, in Expt. *V.* 20 four plants were covered with separate bell-jars standing on large metal plates on the surface of the soil. Each plate closely encircled the stem of the enclosed plant, and all joints were carefully sealed with plasticine. After enclosure in the bell-jar for 27 hours, at 1 p.m. G.M.T. on May 17, 1922 the leaves were bottled. At the same time the leaves of four similar but unenclosed shoots were taken as a control (*V.* 19). Expts. *V.* 22 and 21 (control) were similar, but the enclosed shoots were covered for four days, the leaves being collected at 4 p.m.

<sup>1</sup> These numbers refer to the index numbers in the record book, which have been retained throughout the paper and figures.

G.M.T. on May 21. In Expts. *V.* 24 and 23 (control) the bell-jars were allowed to remain for three days, a wet sponge being placed on each jar to increase the humidity of the air; the leaves were bottled at 1 p.m. G.M.T. on May 24, 1922. The weather during these experiments was dry and hot.

The fourth pair of experiments was on somewhat different lines. The plants were grown in large pots sunk in soil in the open. One plant, 22 in. high (*V.* 27), was removed on July 19, 1924, to a cupboard in a dark room in the experimental greenhouse. The soil was well watered and covered with damp *Sphagnum*, and the pot placed in a vessel containing wet sand. The shoot was covered by a large bell-jar. Under these conditions all parts of the plant might be expected to become practically completely turgid. The leaves were collected on July 22 at 10 a.m. G.M.T., in this case the leaflets only (without the leaf rachis) being taken. A similar potted plant (*V.* 26) still in the open soil in which it was grown was used as a control. During this pair of experiments the weather was warm and humid, much rain falling on July 20 and 21.

The results of these experiments are given in Table II.

TABLE II.

A. *Shoots in still, humid atmosphere :*

Exp.	Leaf 1.	2.	3.	4.	5-6.	PE.	FB.	TB.	Average of all leaves.
<i>V.</i> 20	90.71	—	89.83	—	—	88.16	87.71*	89.21	89.12
22	91.98	90.92	90.31	90.26	—	89.34	88.34	88.56	89.96
24	91.65	90.05	89.13	88.74	—	86.95	86.14†	87.38	88.58
Without petiole									
27	1.45	90.84	90.40	89.87	89.80	88.81	84.21	—	89.24

B. *Control shoots, exposed to ordinary atmosphere :*

<i>V.</i> 19	89.16	—	87.72	—	—	85.75*	86.26	87.50	87.28
21	90.28	89.25	87.83	—	—	86.14	85.87	87.32	87.78
23	89.07	87.81	87.36	86.37	—	84.07†	84.14	85.77	86.37
Without petiole									
26	90.33	89.67	88.57	88.07	87.20	85.29	84.01	—	86.93

C. *Averages and extreme ranges :*

Average									
A	91.45	90.60	89.92	89.62	(89.79)	88.18	86.60	88.38	89.22
B	89.71	88.91	87.87	87.22	(87.19)	85.31	85.07	86.86	87.09
A-B	1.74	1.69	2.05	2.40	(2.60)	2.87	1.53	1.52	2.13
Extreme range	2.91	3.11	3.04	3.89	(2.60)	5.30	4.33	3.44	7.97

Experiments performed in the following pairs :

1st <i>V.</i> 20 and <i>V.</i> 19.	2nd <i>V.</i> 22 and <i>V.</i> 21.
3rd <i>V.</i> 24 and <i>V.</i> 23.	4th <i>V.</i> 27 and <i>V.</i> 26.

In each pair of experiments the water-content of the leaves of the shoots enclosed in still, damp air is greater—on the average by 2.13 per cent.—than in the exposed control shoots. The differences are greater than in our diurnal experiments, though markedly less than the daily fluctuations often found in arid climates (see p. 169). The increase in water-content in damp air occurs in all the leaves of a shoot, but the increments in successive leaves are by no means uniform. On ascending the stem there is a gradual rise in the average increment of water from 1.74 per cent. in the lowest leaf to a maximum of 2.87 per cent. in the partially expanded leaves (PE). Above this there is a fall to the apical bud. If the results of all the experiments are combined, the extreme ranges in the water-content of leaves at the same level on the stem similarly show a rising and falling curve, again with the maximum (5.30 per cent.) in the partially expanded leaves. In other words, the water-content curve for successive leaves is somewhat flatter in humid than in ordinary air (cf. curves in Fig. 2).

These results appear to indicate that under the conditions of our experiments there is during the day-time a general lack of complete turgor in the leaves of exposed shoots, though the leaves themselves may show no visible signs of flaccidity. The water-balance, however, is restored in a still, humid atmosphere. That the successive leaves receive more and more water, the higher their level on the stem, points to a progressive diminution in the degree of turgor as the stem is ascended, the delicate partially developed leaves being the least turgid. This is the case so long as the water balance is not sufficiently adverse to bring about a transference of water from the lower to the upper leaves. An alternative explanation of the observed facts might be that the extensibility of the cell-walls varies with age, the younger leaves with the most extensible walls receiving the greatest amount of water. This may be true in part, but the fall in the increments of water in the apical region, where the cell-walls are the thinnest of all, renders the first explanation the more probable one.

The experiments with *Vicia* yielded very uniform results, but somewhat different results were obtained by a pair of experiments with *Helianthus* (H. 58 and 59, see Table III). Plants were grown in pots under precisely the same conditions as V. 26 and 27, and were about 20 in. high. One plant (H. 58) about 20 in. high was removed to the dark room at the same time as V. 27 and was treated in precisely the same way. The control pot (H. 59), however, was not left in the open, but was transferred to an ordinary dry greenhouse the afternoon before the leaves (laminae only) were collected.

Table III shows that in this case the maximal increase occurred in the lowest leaf, the increment curve falling continuously, rising again only in the terminal bud. At first sight this appears to contradict the results

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ined with *Vicia*. The apparent discrepancy, however, may probably be explained by reference to the data regarding diurnal fluctuations given above. In the climate of Tiflis, Krasnoselsky-Maximov found the greatest nocturnal increment in the lower leaves, the increments decreasing on ascending the stem. This was sometimes the case in our experiments also, but more often the differences between day and night increased from below upwards (cf. Table II). It seems probable that the removal of pot *H.* 59 from the open (where it was sunk in the soil) to the dry greenhouse decreased the available water-supply. Under these conditions the upper leaves may have drawn upon the reserves in the lower leaves, as suggested by Krasnoselsky-Maximov. The fact that the actual water-content of the lower leaves (in *H.* 59) is considerably lower—the maximum being in the terminal bud—than that of the controls in the *Vicia* experiments seems to point to the same conclusion.

TABLE III.

*A. Shoots in still, humid atmosphere :*

Exp.	Leaf 1.	2.	3.	4.	5.	PE.	FB.	EB.	TB.	Averages.
<i>H.</i> 58	91.48	90.23	88.78	88.63	88.13	87.58	87.54	87.26	90.17	88.54

*B. Control plant in greenhouse :*

<i>H.</i> 59	87.48	86.34	85.65	85.97	85.88	85.85	86.87	88.54	88.74	86.57
A-B	4.00	3.89	3.13	2.66	2.25	1.73	0.67	(-1.28)	1.43	1.97

We are probably justified in drawing the following conclusions from the experiments of various investigators on day and night fluctuations and from our own experiments with still, humid air. A diurnal water deficit in foliage leaves during the warmer hours of the day in summer appears to be a more or less general phenomenon. As might be expected, this deficit is more pronounced in dry than in more humid climates. Further, so long as the water deficit does not exceed a certain amount, the resulting loss of turgor is greatest in young, partially developed leaves, decreasing in successive leaves from above downwards. But when this amount of deficit is exceeded it would seem that the younger leaves abstract water from the older, the direction of the turgor gradient being reversed, the lower, oldest leaves now suffering the greatest loss of turgor.

(c) *Experiments with wilting plants.* In order to investigate the relative losses, during wilting, from leaves of different ages, whole plants were made use of. In one experiment (*H.* 15), thirty-two plants of *Helianthus* (5 ft. to 5 ft. 9 in. high) were used, four for each set of determinations. Twenty-eight of these plants, growing in light, sandy soil, were carefully dug up on August 11, 1919, during a spell of hot, dry weather. The soil was gently shaken from the roots, and each plant tied immediately to a garden cane in such a way as to keep the roots above the soil and the

crowns in an erect position. The canes were previously inserted in bare soil, at about four feet apart. Leaves of five different ages were taken, i.e. old mature leaves from node 7 or 8 (counting from below); young mature

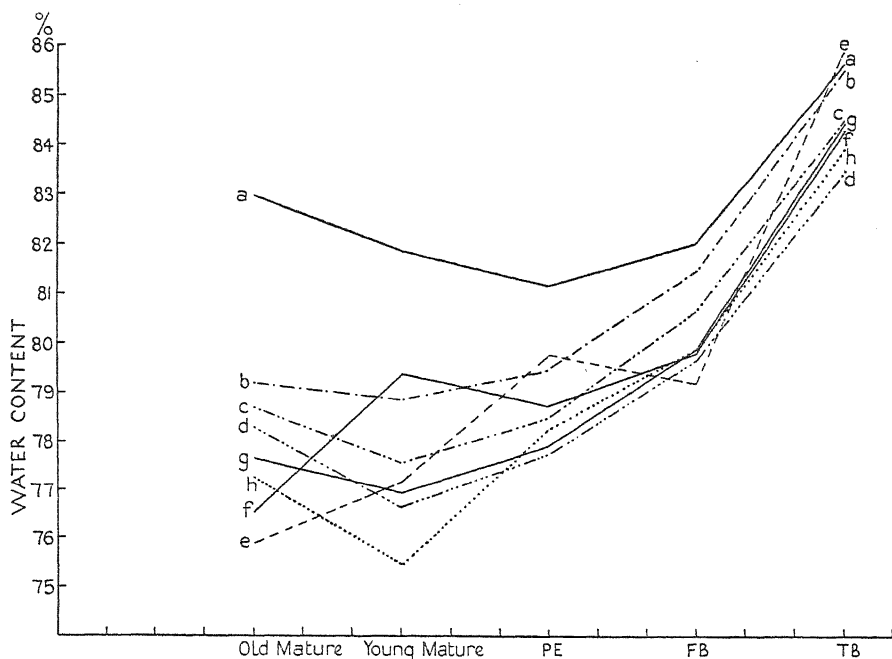


FIG. 3. Curves of water-content of selected leaves from plants of *Helianthus annuus* in various stages of wilting. Four samples were taken from each plant, namely: Old mature leaves from the seventh or eighth node. Young mature leaf from edge of apical rosette (node 11 or 12). PE, partly expanded leaves. FB, leaves just free from the bud. TB, terminal bud. *a*. The control plant while still growing in the soil. Samples taken 11 a.m. *b-g*. Uprooted plants at successive half-hourly intervals, starting at 11.30 a.m.

leaves from the edge of the apical rosette (node 11 or 12); half-expanded leaves (PE); leaves just free from the bud (FB), and the terminal bud itself. At 11 a.m. G.M.T. leaves were bottled from the remaining four plants (controls) still rooted in the soil. At this hour the normal diurnal water deficit in these control plants would be approaching its maximum. Leaves were then collected at half-hourly intervals (beginning at 11.30 a.m. G.M.T.) from the uprooted, wilting plants, beginning with those first dug up. The results are shown in Fig. 3. It is clearly seen that the older leaves lost water more rapidly than those nearer the apex, the greatest loss being during the first half-hour. Subsequently the rate of loss slowed down, but was still fairly regular up to one and a half hours, after which the curves became somewhat more irregular. The mean losses recorded throughout the experiment from leaves of various ages, expressed as percentages of the water-content at 11 a.m. G.M.T. of the control leaves of

corresponding ages, were: oldest leaves used, 6.23 per cent.; young mature leaves, 5.20 per cent.; partly expanded leaves, 3.02 per cent.; leaves just freed from bud, 2.27 per cent.; terminal bud, 1.21 per cent. This rapid loss of water from the older leaves, and the comparatively insignificant loss (in some cases even an apparent gain in the terminal bud, see Fig. 3, E) suggest a transference of water from older to younger leaves (see above). In a similar experiment of shorter duration there was an apparent gain after 50 minutes of 0.29 per cent. in the terminal bud, and of 1.06 per cent. in leaves just free from bud. Older leaves showed a rapidly increasing loss on descending the stem as in Experiment H. 15. These apparent gains of water by the apical region may not, of course, represent actual increases, as the initial water-content may not have been the same in all the plants concerned.

That the lower leaves tend to lose water more rapidly than the upper, when the two sets of leaves are brought into direct competition, is also shown by Experiment H. 43, in which the roots were left undisturbed. A potted plant of *Helianthus* (about 2 ft. 6 in. high) grown in the open, was removed to a dry greenhouse on July 18, 1922, and left unwatered for four days. On July 22, when the leaves were collected, the lower leaves appeared wilted, though the crown was still fresh and erect. On ascending the plant, the water-content of successive leaves showed a steady initial *rise* (instead of the usual *fall*) from 84.33 per cent. in leaf 2 (the lowest still on the plant) to 87.95 per cent. in leaf 6. Above this the water-content gradually fell in the usual manner to a minimum of 82.05 per cent. in leaf 5 (PE), rising again to an absolute maximum of 89.02 per cent. in the terminal bud.

(d) *Water-content of leaves grown under various conditions of illumination.* Previous workers had recorded the fact that 'shade leaves' have a higher water-content than 'sun leaves' of the same species. In order, however, to investigate this question more fully, seeds of *Vicia* were sown in garden soil under cages so constructed as to admit various percentages of normal daylight, without appreciably altering the quality of the light. The percentages admitted to the respective cages were approximately 75, 50, 25, and 10.<sup>1</sup> Normal plants, grown in the open, and therefore receiving

<sup>1</sup> Each cage consisted of a very light wooden framework, to which were nailed thin laths one inch wide, access to the cage being permitted by a door (of similar construction to the walls) on the north side. By placing the laths at appropriate distances apart (i.e. 3 in. in the 75 per cent. cage, and 1 in.,  $\frac{1}{2}$  in., and  $\frac{1}{3}$  in. in the other cages, respectively) the required percentages (approximately only) of incident daylight were admitted. In each cage the top and the four sides (which faced N., S., E., and W.) were similar, so that equal percentages of light were cut off all round. The edges of the laths of the 10 per cent. cage were bevelled on the inside, in order to avoid excluding too great a proportion of oblique light. The cages were painted white outside, but dead black inside, to minimize internal reflection. The dimensions of the 75, 50, and 25 per cent. cages were 5 ft. high, 4 ft. wide, and 2 ft. 8 in. deep (front to back). The 10 per cent. cage was smaller (2 ft. 6 in.  $\times$  2 ft.  $\times$  2 ft.), as the plants had only a limited growth in this percentage of light. To



100 per cent. of the available light, were used as controls. These experiments differed from those described above in that the effect of permanent structural differences due to conditions of growth, rather than mere temporary fluctuations, were here investigated. The results of one set of experiments (V. 1 to 5), in which the leaves were collected between 1 and 2 p.m. G.M.T. on June 4, 1921 (two plants being used for each determination), may be given. The plants, though all of the same age, naturally presented considerable differences. As regards the numbers of leaves, for instance, in addition to the younger stages (PE to TB, see Fig. 4) common to all the plants, those in full light (100 per cent.) had six fully expanded leaves at the time of the experiments; in the 75 per cent. cage five; in the 50 and 25 per cent. cages four each, and in the 10 per cent. cage only three. The results of this set of experiments are shown in Fig. 4. The water-content of the leaves increased fairly steadily as the amount of light received during growth diminished. With 100 per cent. light the average water-content of the leaves was 86.83 per cent.; with 75, 87.68; with 50, 89.13; with 25, 89.75; and the 10 per cent. light, 90.73 per cent. of water. This is equivalent to an average increase in the water-content of about 0.43 per cent. for each decrease of 10 per cent. in the quantity of light reaching the plants during the period of growth. Comparing the extreme cases, i.e. the 100 with the 10 per cent. light plants, the range of average water-content is 3.90 per cent. This is distinctly higher than the temporary fluctuations observed during our diurnal and humidity experiments, though lower than the diurnal differences often found in dry climates (see above).

As in the humidity experiments, the increments of water-content accompanying the progressive diminution of light were distributed unequally in the various leaves. Selecting comparable stages, the extreme ranges in the two sets of experiments are :

	Leaf 1.	2.	3.	PE.	FB.	EB.	TB.
Light experiments	3.00 %	3.88 %	4.72 %	8.27 %	6.06 %	1.01 %	0.65 %
Humidity . . .	2.91 %	3.11 %	3.04 %	5.30 %	4.33 %	—	3.44 %

In both cases the curve has a similar general form, with a maximum range in the partly expanded leaves, but the rise and subsequent fall (to the terminal bud) are more abrupt in the case of the light experiments.

(e) *Shifting of the position of the minimal water-content.* We have seen above that in vigorous, growing plants of *Helianthus*, the absolute minimum is usually in partially expanded leaves (PE), but that in *Vicia* it is somewhat nearer the apex of the plant (FB or even EB). During

secure rigidity the corner posts of each cage were buried deeply in the soil. With cages of these sizes the shadows of the bars, during sunlight, naturally fall across the plants, causing unequal illumination. This in nowise vitiates the experiments, as shade plants in nature are commonly subjected to similar conditions.

our experiments evidence has accumulated that the position of the minimum may vary to some extent according to the amount of water in the plant. With an increasing amount of water a tendency for the minimum to shift

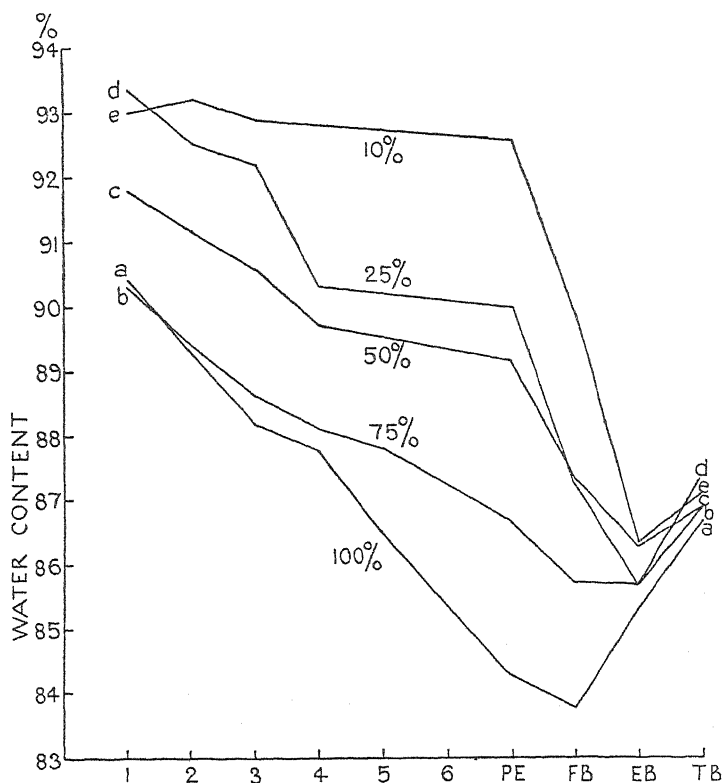


FIG. 4. Water-content curves of leaves from *Vicia Faba* plants grown under various conditions of illumination. The percentages of light for the five pairs of plants taken were: *a* 100, *b* 75, *c* 50, *d* 25, *e* 10. The lettering of the leaves is the same as in Figs. 1 and 2.

nearer the apex is frequently manifested; with a decreasing amount there may be a movement in a basal direction. Correlated with this shifting, the final rise of the water-content curve to the terminal bud becomes less or more pronounced as the case may be.

Compare, for instance, the two composite curves for leaves of *Vicia*, given in Fig. 2. In *V.* (a), constructed from a number of experiments with freely exposed shoots, the minimum occurs in leaves just freed from the bud (FB). *V.* (b), on the other hand, combines the results of experiments with shoots either confined in a still, humid atmosphere or grown in reduced light, both of which conditions increase the water-content of the leaves. In this case the average position of the minimum is in the leaves enclosing the bud (EB). At the same time the curve is distinctly straighter, the final

rise to the terminal bud being barely perceptible. The results of some of the individual experiments combined in the curve *V. (b)* are given in Table II and Fig. 4. In Table II a distinct shift towards the apex is seen in Experiments 20 and 24 (humid air), as compared with the corresponding exposed controls (Experiments 19 and 23). A similar shifting, again accompanied by a less marked rise to the terminal bud, is seen in plants grown in reduced light, as compared with the control plants in full light (Fig. 4). In a companion series of light experiments (*V. 6-10*), conducted later in the same day (6.30 to 7.30 p.m. G.M.T.), the average water-content was somewhat higher than at 1 to 2 p.m. G.M.T. The minimum was now in leaves enclosing bud (EB) in all cases excepting plants in the 10 per cent. cage, in which there appeared to be a further movement of the minimum to the terminal bud itself. A marked shift in *Helianthus* in humid air is also seen in Table III.

Again, in a series of simultaneous experiments (*H. 37-42*) with plants of *Helianthus* of different heights and ages, the position of the minimum varied from leaves about two-thirds expanded (PE) in the tallest plants (1 ft. 9 in.), to leaves just free from the bud (FB) in 3-in. plants, and finally to the terminal bud (TB) in 2-in. plants. The average water-content of the leaves decreased as the height of the plant increased (see p. 168).

Certain other experiments showed a similar shifting of the minimum, but, as might be expected, this was not apparent in all cases; in a few, indeed, the movement, if any, seemed to be in the reverse direction.

At present less evidence is available regarding a definite shifting of the minimum in a basal direction with diminished water-content. The most marked case recorded was that of the wilting experiments shown in Fig. 2. The depression of the position of the minimum is here accompanied by a more pronounced final rise to the terminal bud. In certain old fruited plants of *Vicia*, with a very low average water-content of the leaves, the position of the minimum also appeared to be definitely depressed towards the base of the plant.

#### 4. SUMMARY AND CONCLUSIONS.

A. Under normal conditions, so long as the oldest leaves have not passed their period of maximal functional activity, and new leaves are still being developed from the terminal bud:

1. The maximal water-content is practically invariably in the lowest foliage leaf (in *Helianthus* in the cotyledons).

2. The water-content of successive leaves steadily diminishes with decreasing age and increasing height on the stem,<sup>1</sup> till the minimum is

<sup>1</sup> As found by Mme Krasnoselsky-Maximov, loc. cit.

reached in certain young immature leaves. In *Helianthus* the minimum is usually in leaves which have reached approximately one-third to two-thirds of their full expansion, but in *Vicia* at a somewhat earlier stage, while the leaflets are still folded together.

3. The still younger leaves of the apical region show a steady *increase* in water-content, rising to a secondary maximum in the apical bud.<sup>1</sup>

B. The average water-content of the leaves of a plant decrease with its height and age.

As the oldest leaves die and the youngest approach maturity the water-content curve becomes less steep, and the position of the maximum and minimum less definite.

C. Combining the results of all experiments with normally exposed vigorous plants growing in open soil, the *greatest range* of water-content (i.e. the difference between the absolute maximum and minimum) is found at the same stage of development as the minimal water-content: in *Helianthus* in leaves which have reached about one-third to two-thirds of their full expansion: in *Vicia* both occur in leaves just separated from the terminal bud.

Although the absolute water-content of an organ is not a sufficient criterion of the degree of turgor of its cells, these results indicate greater fluctuations of turgor in the leaves in question than in either the leaves still in the bud, or older, more mature leaves.

Under conditions tending to restore the water balance, the greatest increment of water (and therefore the greatest increase of turgor) occurs in the partly expanded leaves. The same fact is also indicated by the tendency, found in plants either grown or placed under conditions favouring high water-content, for the position of the minimum to shift somewhat nearer the apex of the plant. Thus it would seem that, so far as the water relations of the leaf are concerned, the stage denoted as 'partly expanded' is, in the course of leaf development, the critical one, as it is firstly, the stage of lowest water-content, and secondly, the stage of greatest range in water-content.

D. In simultaneous experiments with plants of various ages (raised from seeds sown at intervals of a week) the water-content of unfolding leaves, *at the same stage of development*, on the whole decrease, with the height of the plant, thus indicating that during what is probably a critical stage of development the upper leaves contain less water than the lower—in other words, their cells are less turgid. Under average conditions in nature the differences may well be greater than those recorded here, for the lower leaves normally develop at a more humid season of the year than

<sup>1</sup> The only previous record of this secondary maximum in the youngest leaves was made by von Hoehnel (4), whose data on this subject seem to have escaped the notice of most subsequent investigators.

the upper. The inference is that the lower water-content at this critical stage in the upper leaves may be a factor in producing the change in anatomical elements in the ascending leaf series known as Zalenski's Law.

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## NOTES.

**THE APHLEBIAE OF HEMITELIA CAPENSIS.**—The aphlebiae of *Hemitelia capensis* have been fully described by many writers and especially by Bower in his volume on 'The Ferns',<sup>1</sup> and by Marloth in the first volume of his 'Flora of South Africa'.<sup>2</sup> They have been well-figured by Schimper,<sup>3</sup> whose figure is copied both by Velenovsky and Bower, and also by Marloth, one of his figures being an excellent photograph of the head of a stem covered by these moss-like pinnae.

When first noticed they were considered to be a filmy fern, from their close resemblance to a *Trichomanes* or *Hymenophyllum*, and were described by Thunberg, in 1800, as *Trichomanes incisum*<sup>4</sup> (Prod. Plant. cap. p. 173). Kaulfuss, in 1824, gave the aphlebiae the name *Trichomanes? cormophyllum* (Enum. Fil. p. 266), for as Sir William Hooker points out (Species Filicum, Vol. I, p. 37), they so closely resemble a filmy fern in a barren state growing parasitically on the tree fern and form a protective covering to the apex of the stem.

These aphlebiae are also of interest to the Fossil Botanist, and Schimper gives a figure of somewhat similar structures in the fossil *Sphenopteris crenata* Lindl. from the Carboniferous period.

Since Bower and Velenovsky have discussed these modified pinnae fully, it is unnecessary to re-cover the ground, but, with regard to their function, Marloth's suggestion that they are equivalent to stipules which, while assimilating themselves, protect the young foliage against the effect of drying winds, &c., is probably correct.

Marloth, from his own observations at the Cape, points out that they are always fully developed before the frond to which they belong makes its appearance, and they lose their chlorophyll and become brown and dry before the next season's aphlebiae begin to show, though the fronds bearing them are still green and vigorous.

On my way from George to Knysna on November 25th, 1930, I visited a fine grove of *Hemitelia capensis* and was fortunate in finding the interesting intermediate pinna here figured. It occurred in the usual position at the base of the frond, but consists of aphlebia on one side of the rhachis of the pinna and normal pinnules on the other side. One pinnule, it will be seen, is partly bilateral in its structure. This example is exactly matched by a specimen preserved in the Kew Herbarium, collected by the Rev. J. Buchanan in Natal (No. 385) in 1870, who also collected a somewhat similar one in 1878, where the lowest member of a large bipinnate frond or

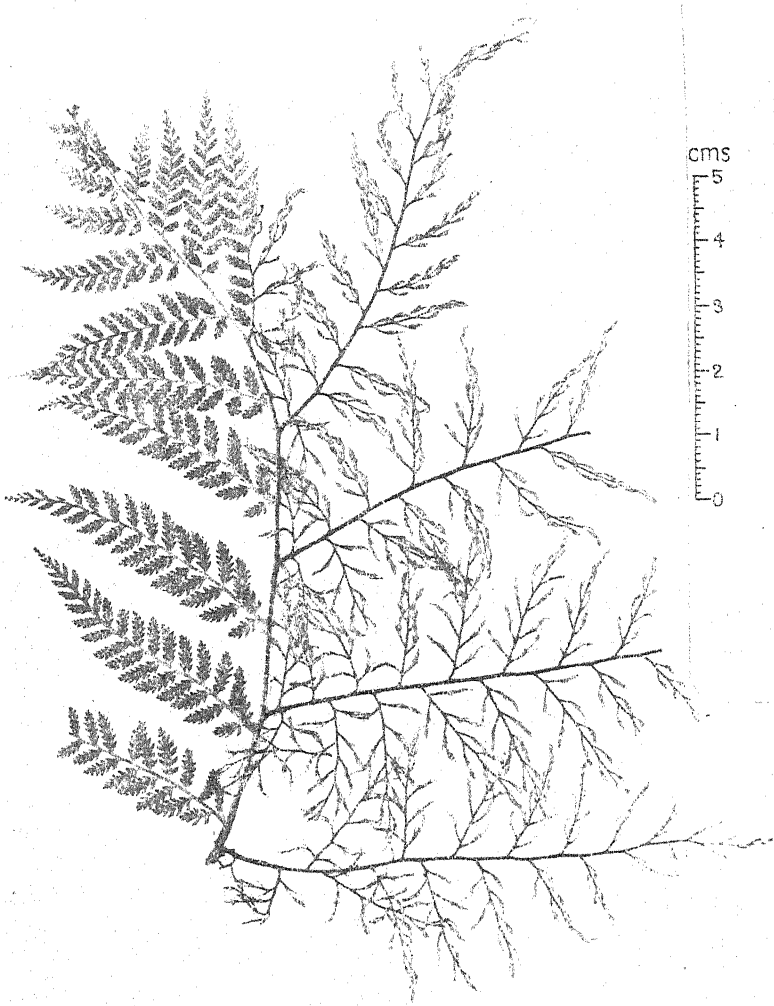
<sup>1</sup> F. O. Bower, The Ferns (Filicales), vol. i, pp. 99-100, Fig. 95. Camb. Univ. Press, 1923.

<sup>2</sup> R. Marloth, The Flora of South Africa, vol. i, p. 75, Figs. 55 A, 55 B, 1913.

<sup>3</sup> Schimper and Schenk, Handbuch der Palaeontologie, p. 143, 1890.

<sup>4</sup> Thunberg, Prod. Plant. Cap., p. 173, 1800. His description runs as follows: 'Trichomanes fronde tripinnata: pinnulis inciso-bifidis setaceis.'

possibly a lateral pinna is aphlebioid, and the rest normal in structure but more delicate than the ordinary pinnae; these examples indicate clearly that the aphlebiae



are modifications of the normal pinnae, and that the basal pair of pinnae have been developed to form a moss-like protective covering to the stem apex.

It is interesting to find in some specimens of *Hemitelia Smithii* that the lowest pair of pinnae are placed near the base of the rachis and are downwardly directed; sometimes the two or three lowest pairs are also inclined downwards and forwards whilst all the other pairs of pinnae spread laterally.

*Cyathea dealbata* shows a somewhat similar arrangement, and the lowest pair of



pinnae thus form a slight protection to the stem apex. *Cyathea Dregii* also shows very definitely the lowest pinnae attached to the base of the rhachis and forming a definite covering closely adpressed to the apex of the stem. In this case, however, though these basal pinnae are quite small, they scarcely differ in general outline from the normal pinnae. Sim, in his Ferns of South Africa (1908), also points out that in *C. Dregii* a pair of small pinnae, similar to the normal ones, frequently occur close to the crown of the stem in this species.

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#### THE CYTOLOGY OF *CALLITHAMNION BRACHIATUM*, BONNEM.

—A paper on the above subject has recently appeared in this Journal<sup>1</sup> the main theme of which is a criticism of some observations made by the present writer.<sup>2</sup> As the results upon which the criticism is based were obtained by a different method of technique, I have repeated some of the work, using the precise method advocated by the writer, namely, the Feulgen reaction. The results which I have obtained differ very slightly from those obtained for this particular plant by using the Heidenhain's haematoxylin method.

Unfortunately the criticism loses value in that the observations on which most stress is laid, and which indeed form the major portion of the paper, were not obtained from the plant in question but from other species.

Assuming that certain nuclear phases, in particular the resting nucleus as described in my paper, are artefacts, it is suggested in the criticism that they were caused by bad fixation. If that is so then the fixative suggested by my critic is equally unsatisfactory, for subsequent examination of material fixed in sublimate-acetic acid reveals, in the same way, the presence of the ring-like structures.

I am indebted to the writer for bringing to my notice a method of technique which seems to be more specific for chromatin than the haematoxylin method. The cytological constituents are more precisely differentiated and the method revealed other phases of nuclear structure which were obscure when Heidenhain's method was employed.

In the accompanying figures it will be seen that the ring-like structures are again visible, particularly in mature reproductive cells such as mature carpospores (Fig. 1). In younger tissues they are also present, though they are not so conspicuous; this is particularly the case in young carpospores (Fig. 2) and young vegetative cells at the tips of the ramuli (Fig. 3).

These cells still retain their power of division, and the ring-like structure is here shown by the Feulgen reaction to be associated with granules suggestive of a reticulum. In the older multinucleate cells of the filaments, however, the nuclear structure is typically reticulate.

<sup>1</sup> Westbrook, M. A., Annals of Botany, vol. xlv, No. clxxvi, Oct. 1930.

<sup>2</sup> Mathias, W. T., The Cytology of *Callithamnion brachiatum*, Bonnem. Publ. of the Hartley Botanical Laboratories. No. 5 1928.

This reticulation is not shown in the mature reproductive cells in which the ring-like structure takes on the 'reddish colouration' with fuchsin-sulphurous acid, and is perfectly homogeneous.

For this reason I cannot accept the writer's suggestion that what I have described is a 'plasmosome nucleolus'. The granules associated with the ring-like structure in the young cells are more suggestive of a prophaseic or an interphaseic condition.

It is stated that nowhere in my paper are vegetative divisions explicitly described. I wish to point out that no pretence of such description was made.



FIG. 1.



FIG. 2.



FIG. 3.



FIG. 4.

FIGS. 1-4. 1. *Callithamnion brachiatum*. Carpospore, resting nucleus. 2. *C. brachiatum*. Nucleus of young carpospore. 3. *C. brachiatum*. Nucleus of apical cell. 4. *C. brachiatum*. Diploid chromosome complex.  $1/12$ th Oil immersion and holos.  $\times 10$ .

If the Feulgen reaction for thymo-nucleic acid is specific, and the method therefore to be regarded as specific for chromatin, I am at a loss to understand why the writer was unable to obtain by its use positive results for *Callithamnion brachiatum*, and yet is apparently enabled to do so by using the haematoxylin method, for in her paper regarding such specificity she definitely states that when applied to the root tips of *Asplenium* 'the chromosomes and reticulum were a bright red-violet'.

The suggestion that the illustrations of diakinesis in my paper are those of a fragmenting nucleolus is one with which I entirely disagree, and would again refer the writer to a similar stage figured by Svedelius in *Delesseria*<sup>1</sup> to which the same suggestion might have been applied were it not for the fact that the nucleolus is still present.

The use of the Feulgen reaction has enabled me to verify the diploid chromosome number in the carpospores (Fig. 4).

Although I have made no observations on *Callithamnion tetricum*, I find great difficulty in accepting the writer's chromosome number for that plant (25-50), as her illustration (Pl. I, fig. 1c) of a spermatial nucleus seems to be typical of an early prophaseic condition; in similar stages of the nuclei in *Pleonosporium Borreri* the variability in the number of granules is such as to range between 5 and 40. It is often difficult to recognize with certainty the most critical stage at which to count the chromosomes, and it is my experience, gained from that of more experienced cytological investigators, that in plants in which the chromosomes are as small as in the Rhodophyceae early anaphase seems to be the most favourable stage at which to obtain an accurate count.

The systematic position of *Callithamnion brachiatum* is a matter of uncertainty and is dealt with in my paper.

<sup>1</sup> Svedelius, N., Über den Generationswechsel bei *Delesseria sanguinea*. Svensk. Bot. Tidsk., Bd. 5, H3, 1911.

With reference to my remarks on *Ceramium rubrum*, I wish to state that I did not *obtain* the results discussed in my paper myself (as I am quoted), but that I noted them when my attention was drawn to the striking similarity in nuclear structure in the preparations of an entirely independent observer.

Regarding the spermatial nuclei the writer's observations are obviously in accord with my own with respect to the ring-like nature of the nuclei, and despite the suggestion that such appearances are artefacts, an acceptable explanation is put forward to explain the existence of these structures in living spermatia. This, to me, appears to be a tacit acceptance of their natural existence.

In my original paper it was admitted from the first that the organization of a ring-like phase in the nuclei was a departure from traditional behaviour of nuclei in the Rhodophyceae, since in other species and genera apart from *Ceramium* I have not observed the peculiarity of structure I have recorded for *Callithamnion brachiatum*; and I may add that until I had examined material prepared and treated in a variety of ways and noted the constant repetition of these curious nuclear manifestations, I had entertained doubts of their validity.

The effect of the recent criticism on the interpretation placed on these nuclear phases, so far from undermining my reading of the facts, has actually afforded an additional means of verification.

W. T. MATHIAS.

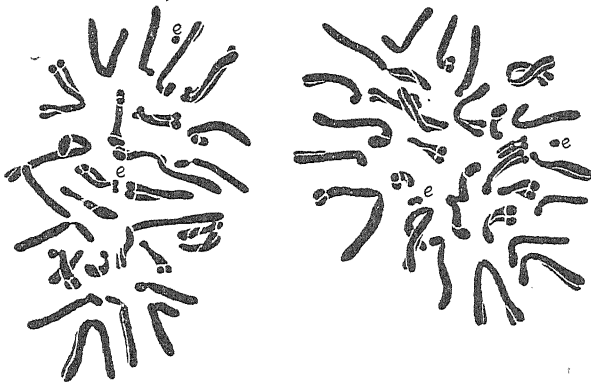
UNIVERSITY OF LIVERPOOL.

**THE CHROMOSOMES OF *RANUNCULUS PARVIFLORUS*, L.**—According to Langlet (Svensk Bot. Tids. xxi. 1-17, 1927) the haploid number of chromosomes in the genus *Ranunculus* is usually eight or sixteen, and less commonly seven, twelve, or twenty-four. Of the thirty-two species (including *Batrachium*) of which the chromosome numbers are cited, five species have seven chromosomes (e.g. *R. acris*) eleven have eight chromosomes (e.g. *R. hederaceum*, *R. bulbosus*, *R. amplexicaulis*, *R. ophioglossifolius*), three have twelve chromosomes (e.g. *R. serbicus*), eight have sixteen chromosomes (e.g. *R. arvensis*, *R. ficaria*), and two have twenty-four chromosomes (*R. muricatus* and *R. trilobus*). *R. parviflorus* is unique among the species investigated in having fourteen chromosomes; this number is met with, however, in the genus *Thalictrum*.

In consequence of the unusual chromosome number, Professor E. J. Salisbury asked me to examine the chromosomes of *R. parviflorus*. Root tips were used and the material was not rich in dividing nuclei. The chromosomes are rather tortuous; and it would appear that at metaphase they never all lie at right angles to the long axis of the spindle simultaneously. Owing to this feature and also to the overlapping of neighbouring chromosomes counts are somewhat difficult; they reveal about twenty-eight chromosomes and thus confirm Langlet's figure.

The tortuous chromosomes render difficult any effort to elucidate their morphology. Attempts to do so indicate that there are three pairs considerably longer than the rest, and that these have median constrictions. About five pairs are relatively short, and of these two or three pairs have sub-terminal constrictions. Of the

remainder, which are of medium length, two pairs have sub-terminal constrictions, and in another pair the constrictions are median. In Langlet's figure 3g no constrictions are shown: this, however, calls for little comment, as they are likely frequently to be concealed owing to the positions the chromosomes assume. At best the description of the constrictions given above can be regarded as only approximately correct.



Chromosome complement of *R. parviflorus*.  $\times 2,700$ . Many of the chromosomes, particularly those more centrally placed, are considerably foreshortened. Chromosomes marked *e* represent the *e* chromosomes of Langlet.

In some metaphases a pair of chromosomes was observed much smaller than the remainder. These are not present in all root tips. They correspond with the *e* chromosomes of which Langlet found from two to ten in cells of one root tip of *R. acris*. It may be pointed out that in both these species the basic chromosome number is seven. Randolph (L. W. Sharp, Introduction to Cytology, p. 402, 1926) has found similar 'diminutive' chromosomes in *Zea Mays*. It seems likely that such chromosomes are fragments detached from the larger chromosomes.

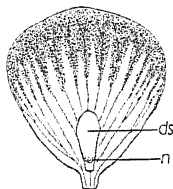
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August, 1931.

**THE GLOSSY PETAL OF RANUNCULUS: A CORRECTION.**—In my paper on 'The Glossy Petal of Ranunculus' (vol. xlii, 1928, of this Journal) *Ranunculus multifidus*, Pursh (*R. delphinifolius*, Torr)—the yellow-flowering aquatic Buttercup of North America—was listed on p. 744 with those species which from the examination of the fresh flowers have been definitely proved to have dull or, as I termed them, mat petals. This now requires correction.

Respecting this species I asked my friend, Professor A. J. Eames of Cornell University, to make the necessary observation on the fresh flower, and he sent in June, 1928, the desired information to the effect that the petal is 'definitely dull'.

Since then Miss M. G. Dudley, while connected with the Botanical Department of the University of Manitoba, kindly made for me some observations in the field on the petals of species of *Ranunculus* to be found growing in the neighbourhood of Winnipeg. In her letter to me of June 8, 1930, she mentioned *R. Purshii*,



Petal (upper surface) of *Ranunculus multifidus*, Pursh,  $\times 2$ , showing distribution of starch (shaded distal area). Note the nectary, *n*, with large dorsal scale, *ds*.

Richards, as having 'decidedly glossy' petals. This species, if not identical with, is very closely allied to *R. multifidus*. I now wrote to Professor Eames asking him to re-examine at his earliest opportunity the petals of *R. multifidus* in his district to see if he could confirm his former observation.

On July 23 of this year he replies as follows: 'I hope you can stand the shock as I have had to! I have at last found flowers of *Ranunculus delphinifolius* [= *R. multifidus*], and they are part dull and part glossy, the proportion varying somewhat from half or a little less dull to two-thirds or perhaps rarely three-quarters dull. Now you wonder as I did, how it happened that I told you (and you printed) that the petal was definitely dull. I hardly know. But I have not been able to get flowers where I saw them before, and the fact probably is that there was a very high percentage of surface dull in that colony. Further, the glossy area, though *definitely* glossy, is not nearly so much so as in the petals *R. bulbosus* and *R. acris*. This [giving a rough sketch] is a fair example of the condition. It is, you see, much as Miss Dudley reported for *R. Purshii*. Well, needless to say, I am much chagrined at having led you astray (tho' I think I must have seen material with at least seven-eighths dull instead of what this year seems to be the condition, a half to two-thirds dull).'

From the character of the nectary I fully expected *R. multifidus* to have a mat petal (loc. cit., p. 746, para. 1), and in my original letter to Professor Eames asking him to make the observation I conveyed as much. This may have led him to make a more cursory examination than he would otherwise have done.

So far in my investigation of the genus this species is unique in having gloss associated with a nectary possessing a dorsal scale. In other glossy yellow Buttercups when a scale is present it is ventrally placed. The style of nectary possessed by *R. multifidus* is characteristic of a number of white Buttercups,\* e.g. *R. aconitifolius*, *R. amplexicaulis*, *R. parnassifolius*, *R. glacialis*; and also of the mat yellow species, *R. gramineus*.

An examination of the spirit material of *R. multifidus* kindly supplied me by Professor Eames reveals a considerable variation in the starch-containing surface of the petals. Assuming that the starch and the gloss coincide, then, this petal is

\* Petals of white species of *Ranunculus* are never glossy as far as my knowledge extends.

exceptional in not only having a large basal non-glossy area, but also in showing variability in the extent of this. Can the explanation be that in this species the gloss is tending to disappear; or possibly, though less likely, that it is in the process of evolving?

In glossy petals of *Ranunculus* the line separating the basal mat portion from the upper starch-containing area is not even; but inclined to be zigzag, through the tendency of the starch to extend downwards far her between the veins (vascular bundles) than along them. Or to express it in the opposite way, the basal starch-free portion tends to encroach on the glossy area along the veins.

In *R. multifidus* these encroachments, as shown in the accompanying text-figure, are very much accentuated (though, oddly, the starchless intrusions are here between and not along the veins). Thus, though the starch may extend half-way down this petal, yet through these intrusions the glossiness of its upper part will be less apparent than it otherwise would be. This may account for Professor Eames's remark in his letter just quoted that the gloss is less marked than in such petals as *R. bulbosus* and *R. acris*.

*R. multifidus*, then, offers to be of particular interest in the study of the evolution of gloss and starch in the petal of the genus. From seed collected and sent me by Professor Eames I am hoping to raise plants to the flowering stage, and thus obtain first-hand evidence. Seeds have already germinated.

J. PARKIN.

BLAITHWAITE,  
WIGTON,  
CUMBERLAND.  
November, 1931.

## Two Little-known Genera of Green Algae (Tetrasporidium and Ecballocystis).<sup>1</sup>

BY

M. O. P. IYENGAR.

With Plates VII and VIII and nine Figures in the Text

PART I. *Tetrasporidium javanicum*, Moebius.

THIS alga was described by Moebius from Java in 1893 (11, p. 122), and was again collected by Massart in 1896 (cf. 19, p. 32), but has not since been recorded either from Java or from any other part of the world. In the fairly detailed account published by Moebius many interesting features are, however, overlooked, presumably owing to the scantiness of the material at his disposal. Moreover, his record of the occurrence of sporangia is based on a misinterpretation. A certain amount of interest attaches to this alga, owing to the uncertainty as to its exact systematic position. Wille (20, pp. 30 and 31) placed it in the Tetrasporaceae under 'Wenig bekannte oder unsichere Gattungen', and the same procedure was adopted by Printz (13, p. 79) in 1927, owing to the absence of any further information about this alga. Blackman and Tansley (1, p. 241) also refer it to Tetrasporaceae, while Chodat (4, p. 112) suggests inclusion in the genus *Tetraspora*.

*Tetrasporidium javanicum* is not uncommon in Madras, and advantage was taken of the large amount of material available to make a careful study of the alga, both in the living and preserved conditions, in the hope of throwing more light on its structure and development, as well as on its systematic position.

Moebius records the alga from a river. In Madras it generally occurs in the small pools formed during the rainy season (October to December) growing on aquatics, such as *Monochoria hastataefolia*, Presl. (*M. hastata*, Solms), and certain grasses; to these it is attached by a broad base, beyond which the thallus floats out on the surface of the water. It was also once found stranded by the current on the sandy banks of the river Nagari in South India,<sup>2</sup> along with *Hydrodictyon reticulatum*.

<sup>1</sup> From the Department of Botany, East London College (University of London).

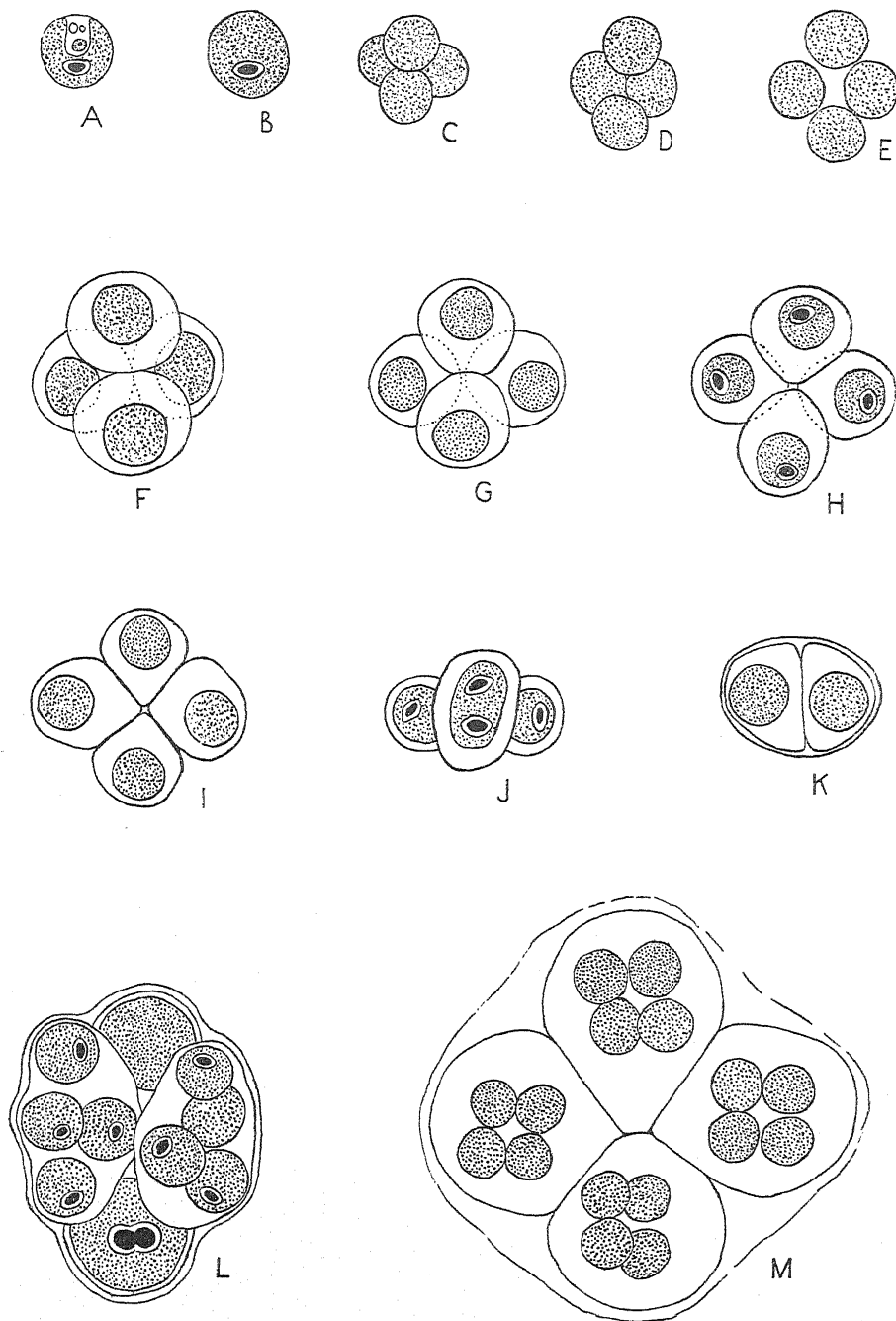
<sup>2</sup> I am indebted to Mr. N. Venkatanathan for providing me with material from this locality.

The thallus, when fully grown, is a thin, flat, more or less circular, gelatinous expanse of pale green colour which may reach a diameter of 12–13 cm. (5 inches) (Pl. VII, Fig. 1). The youngest stages seen were about the size of a pea and roughly spherical or oblong in shape. Moebius's specimens must have been young or fragmentary, since he puts the maximum size of the thallus at 2 cm.; he himself, however, suggests that these may have been merely fragments. The young stages found by me were apparently quite solid and contained numerous cells.

When a mature thallus is examined under the lower power of the microscope, it presents a net-like appearance with numerous, round or elliptic, and often large and irregular perforations with a smooth or lobed margin; these perforations vary from a minute hole to a large irregular aperture (Pl. VII, Fig. 3), and their edge is more clearly seen on staining. The numerous cells are embedded in a gelatinous matrix within which at first sight they appear to form a single layer. Closer examination, however, discloses that the thallus really consists of two superposed layers of mucilage, each provided with an independent series of perforations, the two layers being connected with each other at various places by irregular gelatinous processes (cross-connexions) in a rather haphazard manner (Pl. VII, Figs. 2, 4). The impression obtained is that of overlapping of different parts of the thallus as a result of careless mounting, but, if specimens are floated out on a slide from a dish of water, just the same appearance is observed. At the edges of undamaged thalli, it can be seen that the upper and lower layers are continuous with one another, but the edge is not sharply defined and seems to form part of a curved surface. The thallus as a whole appears indeed to represent a hollow sack with a one-layered wall broken by numerous perforations, overlying parts of the wall being joined by the cross-connexions just referred to. This structure is, however, obscured and rendered rather difficult to decipher owing to the numerous perforations and the many connecting branches between the opposite surfaces of the thallus. The way in which the hollow state is arrived at has not been determined with certainty, but it seems probable that the originally solid thallus becomes hollow and that the cells become distributed in a single layer within the mucilage enveloping the hollow. The origin of the perforations and the cross-connexions is dealt with below.

The individual cells closely resemble those of a globular *Chlamydomonas* in structure, the general shape being rounded to rounded-oval; the diameter of the protoplast of young cells is 7–9  $\mu$  and of fully grown ones 10–11.5  $\mu$ . Each cell has a small round nucleus and a bell-shaped chloroplast with a large median elliptical pyrenoid lying near the posterior end, while in living specimens two contractile vacuoles (max. diam. 1.75  $\mu$ ), pulsating alternately, were distinctly visible at the anterior end (Text-fig. 1, A). There is no eye-spot. Although various stains and reagents





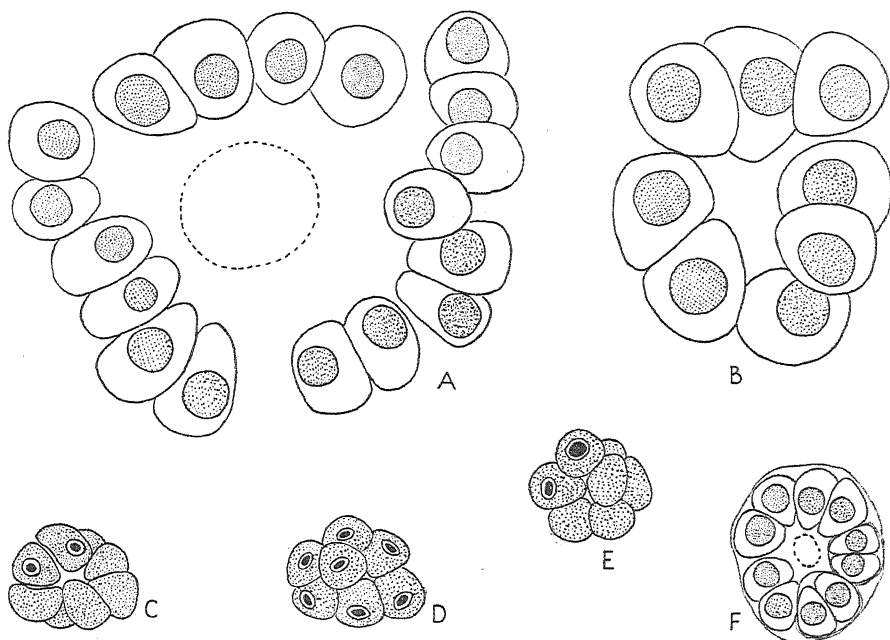
TEXT-FIG. 1. *Tetrasporidium javanicum*, Moeb. A, single cell; B, full-grown cell preparatory to division; C-E, division in two planes, leading to crosswise arrangement shown in I; J, a case of arrested division with one of the cells elongated and with two pyrenoids; K, division into two; L, M, mother-cell walls of three generations intact. Pyrenoids black. A  $\times 920$ ; B-M  $\times 1,050$ .

(including iodine, safranin, fuchsin, methylene blue, gentian violet, haematoxylin, toluidine blue, and eosin) were used, no pseudocilia could be found. In place of the firm cellulose wall of a *Chlamydomonas* there is around each protoplast a thick, hyaline, but fairly consistent gelatinous envelope which can be clearly seen only after treatment with a dilute aqueous solution of safranin, methylene blue, toluidine blue, or Delafield's haematoxylin (Text-fig. 1, F-K). The general mucilage of the thallus is mainly composed of these envelopes, though there is also a certain amount of homogeneous intervening mucilage, which is seen clearly at the edges of the perforations. There are no protoplasmic connexions between the cells.

The cells multiply by division, either into two (Text-fig. 1, K) or, more commonly, into four. In the latter case the division appears to be successive, first into two and then into four, and most commonly the four daughter-cells lie in the same plane which is parallel with the surface of the thallus. Not infrequently, however, the two successive divisions take place in planes respectively parallel and at right angles to the surface of the thallus. If the plane of the first division is parallel to, and that of the second perpendicular to the surface, we obtain groups of cells exhibiting a crosswise arrangement in two planes (parallel to the surface of the thallus), i.e. there is a pair of upper and a pair of lower cells, the lower alternating with the upper (Text-fig. 1, C, D, F-H). Occasionally one finds one of the two daughter-cells of the first division undivided, such cells being elongate with a pyrenoid at each end (Text-fig. 1, J). If the plane of the first division is perpendicular to the surface and only one of the two resulting daughter-cells then divides in a plane parallel to the surface, three of the daughter-cells lie in one plane and the fourth in another. In such cases, especially if the mucilage be a little displaced, the cells appear to have a tetrahedral grouping, but close scrutiny supports the view of the origin of such groups in the way described. Sometimes division into eight is observed, the daughter-cells exhibiting no very regular arrangement, though more or less radially grouped (Text-fig. 2, C, D, E). Division into eight is, however, comparatively rare, and appears, as explained below, to be connected with the formation of the perforations in the thallus. In all cases, the daughter-protoplasts secrete a gelatinous wall distinct from that of the parent-cell, and the new envelope can be clearly distinguished inside the latter. When the daughter-cells divide again the same process is repeated and, in stained preparations, the envelopes of successive generations can often be clearly, though faintly, seen (Text-fig. 1, L, M).

When division into four takes place, the daughter-protoplasts at first lie close together but, as they become surrounded by their own envelopes, they get pushed apart. This appears to be due to the fact that the secretion of the gelatinous envelope takes place more actively on the side of the

cell towards the centre of the group, so that the individual protoplasts come to lie near the outer boundary of each group (Text-fig. 1, F-I, K). When the two divisions have been in different planes, and the pairs of daughter-cells are placed crosswise, the cells appear gradually to become disposed in one plane, though often showing a slight overlapping of the inner edges of their gelatinous walls. The four cells then appear to be separated by



TEXT-FIG. 2. *Tetrasporidium javanicum*, Moeb. A, B, F, beginning of a perforation after division into eight cells; C-E, division into eight. Pyrenoids black. A-E  $\times 870$ ; F  $\times 440$ .

partition-walls which are arranged crosswise, and such groups can be found abundantly within the thallus. In the centre of each group, there often appears to be a small approximately square space, where the four 'partitions' meet, reminding one of the intercellular spaces of higher plants (Fig. 1, H, I). But these apparent spaces in *Tetrasporidium* are not due to splitting, but to the overlapping of the wall of the cells of each group. It has not been possible to ascertain the course of events in those cases in which the products of division show a pseudo-tetrahedral grouping, but it is to be presumed that here also all the cells come to lie in one plane.

When division into eight takes place (Text-fig. 2, C-E), the enlargement of the daughter-cells after they have formed their gelatinous walls appears invariably to lead to the formation of a tiny rupture between the converging inner ends of the cells. With the further division of the latter, these small spaces are gradually converted into large holes. It seems that

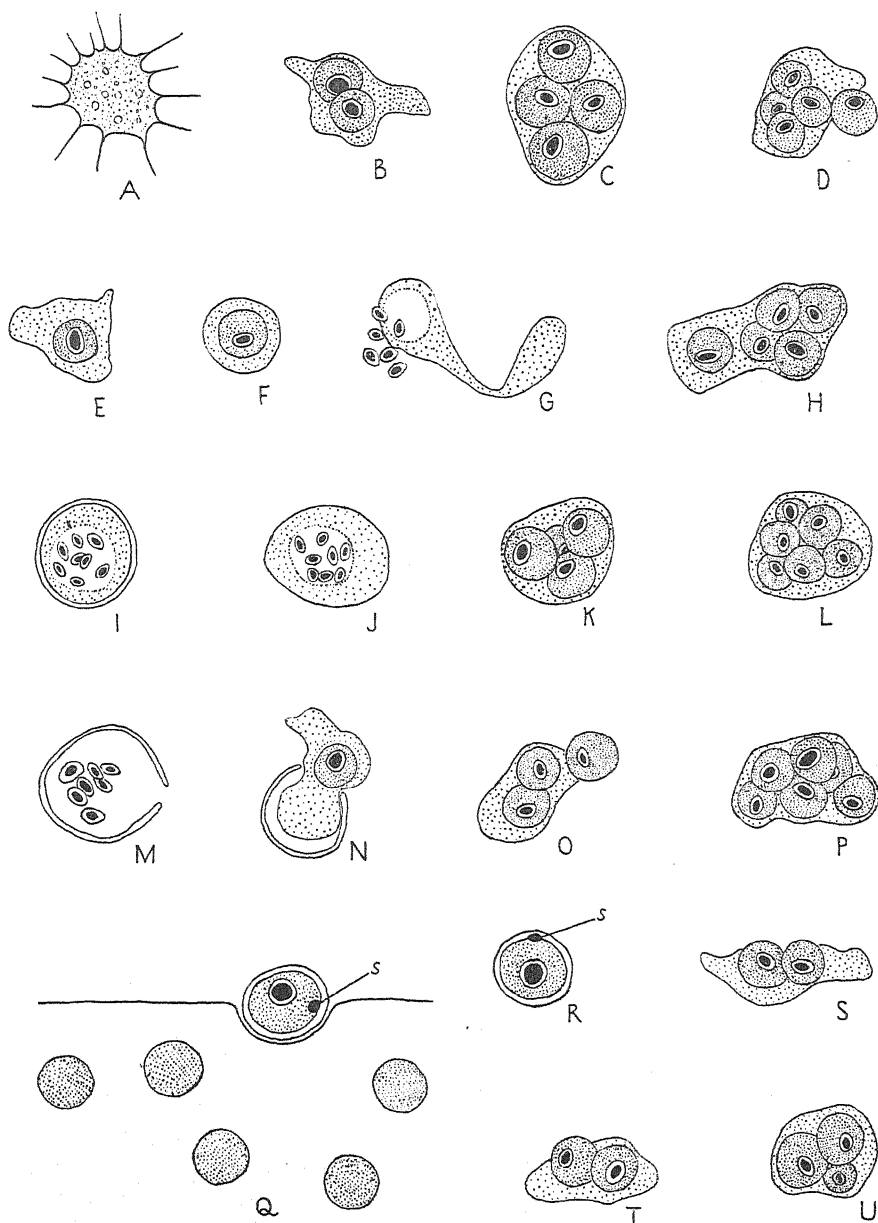
the simultaneous enlargement of a considerable number of cells all converging to a single point sets up local strains leading to the rupture of the thallus at such places (Text-fig. 2, A, B, F). The cells round the edges of the perforations are always more densely grouped than elsewhere.

*Origin of the cross-connexions between the two layers of the thallus.* From the edges of the perforations thus formed there grow out small lobes which, increasing in length, gradually extend across the perforations and finally fuse with some other point on the margin. In other cases these lobes may grow towards the opposite surface of the hollow thallus and become joined up with it at their tips. Similarly, large lobes may arise at any point from the inner surface of one of the two layers of the thallus and, growing towards the other layer, may unite with it at various places in an intricate manner. There does not appear to be any order or regularity in the formation of these cross-connexions, with the help of which the opposite surfaces of the thallus are closely knitted together. The thallus thus superficially resembles an *Ulva*, in which the two layers have split apart and become irregularly perforated, although connected together by numerous cross-branches.<sup>1</sup>

*Reproduction.* Although the living alga was kept under prolonged observation at Madras, no motile reproductive cells of any kind were noticed. The presence of contractile vacuoles in the ordinary cells, however, renders the occurrence of motile stages probable. Nor were any resting spores seen, and the way in which the alga tides over the dry season when the pools dry up still remains to be established. *Palmodictyon viride*, Kütz., which shows a certain resemblance to *Tetrasporidium*, is known to form hypnospores (16, p. 246), and, here too, swarmers have not been observed.

Moebius (11, p. 122) records sporangia whose contents, as a result of successive division, give rise to a number of spores. Structures similar to those described and figured by Moebius as sporangia occurred frequently in my material, but I am of opinion that they are due to a small protozoon parasite which is found in the gelatinous matrix of the *Tetrasporidium*. The organism in question is no doubt a *Vampyrella*, and is possibly identical with *V. lateritia* (Fresen.), Leidy, but the material was inadequate for an accurate determination of the species. The individuals observed within the mucilage of the alga were brownish-red in colour and approximately spherical, the protoplast being produced into numerous delicate

<sup>1</sup> A small unicellular epiphyte was found growing attached to the surface of the thallus, the spherical cells being about the same size as those of the *Tetrasporidium*. These cells had a well-defined firm cell-wall, and their contents resembled those of a *Chlamydomonas*, though less granular than those of the *Tetrasporidium*, while the large pyrenoid was rounded and not elliptic; there was a very definite eye-spot. This epiphyte was always seated on the outer surface of and opposite a depression in the mucilaginous matrix of the *Tetrasporidium*, being often attached to the edges of the perforations (Text-fig. 3, Q, R). This form resembles *Malleochloris sessilis*, Pascher (Süsswasserfl., iv, 1927, p. 480), but does not possess the short gelatinous attachment of the latter.



TEXT-FIG. 3. A, *Vampyrella* from the mucilage of *Tetrasporidium*; B, C, E, F, H, K, L, P, U, the parasite with ingested cells of *Tetrasporidium*; D, O, S, T, parasite about to ingest a further cell; G, ejection of undigested portions of algal cells; J, parasite with the algal cells (apart from the pyrenoids) completely digested and about to encyst; I, encysted parasite; N, the parasite escaping from its cyst-wall with an algal cell already ingested; M, empty wall of cyst from which the parasite has escaped leaving undigested pyrenoids behind; Q, R, an algal epiphyte (*Malleochloris*, sp.?) on *Tetrasporidium* showing the eye-spot (s). Pyrenoids black. A  $\times 810$ ; B-U  $\times 920$ .

pseudopodia (Text-fig. 3, A). These amoeboid individuals were in the living material seen to attack cells of the alga (Text-fig. 3, D, O, S, T) which became engulfed within the protoplast of the parasite (Text-fig. 3, B-F, H) and were then slowly digested, the undigested portions often being finally extruded from the body of the parasite (Text-fig. 3, G). The pyrenoids could be detected for a long time after the algal cells had been engulfed by the parasite and did not appear to undergo digestion (Text-fig. 3, I, J), since they formed the principal part of the extruded undigested material (Text-fig. 3, G). Usually the remains of a number (up to eight or more) of algal cells were to be found within the body of the parasite. It is not clear whether this is due to the ingestion of successive algal cells or to a number of the parasitic protoplasts uniting to form a plasmodium, as is undoubtedly often the case. It is also possible that the algal cell continues to live for some time within the body of the *Vampyrella*, and may even divide during that period.

Such stages of the parasite with a number of included algal cells soon secrete a definite enveloping wall. Owing to the practically unaltered condition of the pyrenoids, the contained algal cells still appear to be intact, and in this condition the encysted parasite, with its included algal cells, superficially resembles a sporangium with a number of spores inside (Text-fig. 3, C, K, L). The semi-transparent granular body of the parasite surrounding the algal cells it has encompassed resembles a peripheral layer of protoplasm around the 'spores' of the 'sporangium'. Moebius evidently misinterpreted these stages of the parasite as sporangia of *Tetrasporidium*, which are stated to possess a certain amount of periplasm; this latter is, however, actually the protoplasmic body of the parasite.

*The systematic position of Tetrasporidium.* Since the cells do not possess any pseudocilia, the suggested inclusion of the alga in the genus *Tetraspora* (cf. Chodat and others) cannot be supported. In fact, for this reason, it cannot be included in the Tetrasporaceae and must find a place in the Palmellaceae, an artificial family established for those palmelloid Green Algae that lack the pseudocilia of the Tetrasporaceae. It does indeed appear to come rather close to *Palmodictyon viride*, Kütz., which it resembles in the cells having gelatinous walls which retain their individuality for a long time and in the formation of anastomosing lobes on the thallus. The apparent absence of motile reproductive stages in *Tetrasporidium* constitutes another point of resemblance to *Palmodictyon viride*. There is some uncertainty about the nature of the chloroplasts in *Palmodictyon*, since Lemmermann (10, p. 35) describes the cells as containing several curved disc-shaped chloroplasts without pyrenoids, whilst according to West (16, p. 240), in *P. varium* (Naeg.) Lemm. (*Palmodactylon varium*, Naeg.), there is a parietal irregularly lobed chloroplast.

Moebius largely based his genus on the occurrence of sporangia with

a peripheral formation of periplasm, a feature which has been shown above to be due to a parasitic organism. But *Tetrasporidium* exhibits a number of other peculiarities that for the present justify its retention as a separate genus. In its cell-structure *Tetrasporidium* clearly resembles a *Chlamydomonas*, differing only in the absence of an eye-spot and of cilia and in the consistency of the wall. In the possession of the two contractile vacuoles it shows a closer relation to motile types than do most of the other members of the Palmellaceae, although in *Asterococcus*, Scherffel, both contractile vacuoles and eye-spots have been recorded (18, pp. 38 and 40).

The emended diagnosis of the genus rendered necessary by the facts established in this paper is as follows:

Thallus macroscopic, attached by a broad base to other aquatics and forming a gelatinous two-layered expanse, the two layers separated by a hollow, but continuous at the edges, each layer provided with numerous perforations of diverse size; opposite edges of the perforations, as well as the two layers of the thallus, joined by numerous cross-connexions formed by the protrusion of lobes which ultimately anastomose with other parts of the thallus; cells numerous, somewhat remote from one another, embedded in a gelatinous matrix formed largely by the gelatinous cell-membranes, without any protoplasmic connexions, each with a posterior bell-shaped chloroplast containing a single pyrenoid and with two contractile vacuoles at the anterior end, but without cilia, pseudocilia, or eye-spot; cell-wall gelatinous, but fairly consistent, the walls of successive cell-generations remaining distinct for some time and clearly seen after staining; cell-division into two, four, or eight; zoospores, gametes, and resting-spores unknown.

*Geographical distribution*: Java, S. India.

## PART II. The genus *Ecballocystis*, Bohlin.

This genus was established by Bohlin in 1897 (2, p. 7) for an alga which occurred in collections from Rio Grande do Sul in Brazil, and which he described under the name of *E. pulvinata*. It formed small macroscopic cushions, attached to stones in a river, composed of numerous elongated cells arranged in a characteristic manner. Bohlin describes this form as follows: 'If one starts with a *single* cell, the mode of division is as follows. The mother-cell divides first by a transverse wall into two daughter-cells, which in the course of their growth very soon become obliquely disposed with respect to each other. Often a second division may take place before the bursting of the membrane of the mother-cell, but usually the membrane breaks already after the first division. In the latter case the upper daughter-cell is pushed obliquely upwards and becomes fastened at its base to the wall of the mother-cell by a cone-shaped secretion from the cell-wall which occupies the free space in the lower part

of the cell. Each daughter-cell thereupon divides with repeated rupture of the respective mother-cell walls and attachment of the upper daughter-cell to the same. Generally the divisions take place somewhat more rapidly on one side of the colony. All cells are, however, capable of division, and displacement of the cells takes place in several directions. In this way there arises a slightly branched colony in which one can observe the ruptured membranes of several generations. The remains of the older membranes seem to gelatinize, and in this way even macroscopically visible cushion- or mat-like coverings are formed on the substratum. The chromatophores are pure green and contain starch; for the rest nothing of their structure was to be seen in the available material. The membrane showed no cellulose reaction.'

The essential features of the alga are the oblique arrangement of the daughter-cells, the attachment of the latter to the walls of the mother-cells by a basal secretion of mucilage, and the dendroid colonies with their false branching. In 1903 Yendo (21) referred three marine algae to the genus *Ecballocystis* but, as pointed out by Printz (13, pp. 78, 79), these should be placed in the genus *Collinsiella*. In 1917 Fritsch (7, pp. 494-502) described from S. Africa two species of *Ecballocystis*, *E. ramosa* and *E. simplex*, which are referred to in greater detail below. The writer has met with abundant material of species of *Ecballocystis* in various localities in Southern India, the majority of which differ in important respects from the forms hitherto described. It is thus evident that the genus has a wider geographical distribution than has so far been recognized, and that it displays a considerable range in morphological structure. In the following matter the individual species are first considered separately, and this is followed by a general discussion of the genus.

*Ecballocystis Fritschii* sp. nov. (Text-fig. 4).

The thalli of this alga are small, irregularly rounded, lobed masses, sometimes somewhat cylindrical, about one-eighth to one-third of an inch wide and about one-eighth to one-third of an inch high. They were found attached to the rocky bed of a small stream. The masses were compact enough and sufficiently firmly attached to resist the force of the fairly strong current. Some of the material was kept under observation in a living condition, while the rest was preserved either directly in 4 per cent. formalin or after fixing in Flemming's weaker chromo-acetic solution and subsequent washing.

The alga resembles *E. pulvinata*, Bohlin, in a general way in the structure of the thallus and the disposition of the cells. Since, however, Bohlin's description and figures do not afford any data as to the cell-structure, the degree of correspondence between the two forms cannot be fully estimated.





TEXT-FIG. 4. A-H, M-O, R-U, *Ecballocystis Fritschii*, sp. nov. A-E, cells showing chloroplasts; F, cell showing only the nucleus; G, division of protoplast; H, formation of two daughter-cells inside mother-cell wall; M, N, O, S, formation of four daughter-cells (in N seen from above); R, T, U, parts of mature colonies, showing dendroid branching. I-K, L, P, Q, *E. Fritschii* var. *pulneyensis* var. nov.; I-K, cells with chloroplasts; L, two daughter-cells at apex of ruptured mother-cell wall; P, empty mother-cell wall; Q, a single daughter-cell remaining at the base of the mother-cell wall. Pyrenoids black. L, P, Q  $\times 450$ ; the rest  $\times 890$ .

*Cell-structure.* The cells are elliptic oblong with broadly rounded ends. There is a single central nucleus suspended in the vacuole by radiating cytoplasmic threads (Text-fig. 4, F) and two, four, or eight, more or less curved, discoid, parietal chloroplasts, each containing a single pyrenoid (Text-fig. 4, A–E). The younger cells have two chloroplasts only (Text-fig. 4, A), but in most of the older ones there are four (Text-fig. 4, B, C) and, in a few of the fully grown cells, eight (Text-fig. 4, D, E). In cells with four chloroplasts the latter are arranged in two pairs in the upper and lower halves of the cell respectively, the two chloroplasts of each pair standing opposite to each other; the two pairs of chloroplasts may lie in the same plane (Text-fig. 4, B) or be placed at right angles to one another (Text-fig. 4, C). When there are eight chloroplasts in a cell, they are sometimes arranged in four superposed pairs (Text-fig. 4, E), but the arrangement is not always so regular; often one sees five chloroplasts at one focus and three below (Text-fig. 4, D). The older cells are full of starch. The pyrenoids are very small and inconspicuous in the older cells, but somewhat larger and more distinct in very young cells, especially in those containing two chloroplasts only. There is a thin but definite starch sheath around the pyrenoid. Staining with iron-haematoxylin or Delafield's haematoxylin brings out nuclei, chloroplasts, and pyrenoids clearly. The cell-wall is thin and firm, but very slightly thicker at the basal pole, a fact especially obvious after the rupture of the wall has taken place; the wall is not coloured blue by chlor-zinc-iodide. It envelops the contents closely at all points. The mucilaginous matrix, within which the cells of the colony are embedded, readily takes up various stains such as methylene blue, congo red, &c.

*Cell-division and colony-formation.* Division of the protoplast takes place obliquely into two (Text-fig. 4, G), and a new wall is soon formed around each of the products, with the result that two somewhat obliquely placed cells are seen inside the membrane of the parent-cell (Text-fig. 4, H). As the daughter-cells increase in size, the latter at first becomes distended, but ultimately undergoes rupture at the apex. In the course of further growth, the daughter-cells come to lie at approximately the same level near the mouth of the ruptured membrane of the parent-cell, as shown in Text-fig. 4, R, U. The daughter-cells in their turn divide, and form grand-daughter-cells whose enlargement leads to a similar rupture of the daughter-cell membranes, and this process continues until a fairly big aggregate is formed. The cells appear to secrete a certain amount of mucilage, while at the same time the membranes of the older cells gradually undergo gelatinization, so that an extensive mucilaginous matrix is produced with a large number of cells embedded in it. Within the gelatinous matrix the remnants of the mother-cells of several generations can always be detected (Text-fig. 4, U). If a small piece of the thallus is stained on the slide for

a few minutes with methylene blue or congo red and gently pressed under a cover glass, the arrangement of the old mother-cell walls and the dendroid nature of the thallus can be clearly made out (Text-fig. 4, R). The thallus is seen to be built up of successive 'branchings', each resulting from a cell-division. The conical gelatinous processes at the base of the cells described by Bohlin in *E. pulvinata* are lacking in this form. In Bohlin's form, moreover, the lower ends of the old mother-cell walls are broadly rounded (2, Figs. 2, 3, and 4), whilst in *E. Fritschii* they are funnel-shaped and somewhat constricted below.

Gelatinization of the wall of the mother-cell always seems to commence at the top, where rupture takes place, and from there it spreads slowly to the rest of the wall. The slightly thickened basal portion of the wall is the last to become completely gelatinized. The process of gradual gelatinization from the apical region downwards is readily followed. If a piece of the thallus is stained with dilute aqueous solution of toluidine blue, the gelatinized portion of the wall is stained blue, while the ungelatinized firmer portion becomes bluish-yellow. The upper thinner portion of the ruptured wall is more easily distended by the enlarging daughter-cells than the lower, and this results in the funnel-shaped form previously referred to. The upward movement of the daughter-cells is, moreover, probably in part due to the pressure of the elastic lower part of the wall upon them, although secretion of mucilage inside the mother-cell wall may also play a part. It may be pointed out that the upward movement of the daughter-cells is not due to migration of the protoplast inside the cell, as suggested by Davis (6, p. 377) in *Euglenopsis*, but that the movement is purely passive.

During this passive transference towards the aperture of the ruptured mother-cell wall, the lower of the two daughter-cells glides past the lower end of the upper one and becomes placed alongside of the upper cell at the mouth of the funnel, though very slightly below it. As a consequence both daughter-cells come to lie near the edge of the ruptured mother-cell wall and thus, as the gelatinous thallus develops with repeated cell-division, all the cells become arranged near the external surface. Occasionally it happens that one of the two daughter-cells remains at the base of the parent membrane (Text-fig. 4, R). Such cells do not appear to divide further. Microtome-sections of the thallus likewise show that practically all the living cells are placed near the outer surface with their longitudinal axes roughly at right angles to the latter, while the inner portion of the thallus is occupied mainly by the empty cell-walls and the mucilaginous matrix with a sprinkling of isolated cells (Pl. VIII, Fig. 8). Some of the fully developed thalli are even found to be hollow in the centre, a fact visible with the naked eye. In the detailed structure of the colony therefore this species contrasts markedly with *E. pulvinata*.

*Reproduction.* The cells usually divide into two daughter-cells only,

but division into four is not uncommon. The daughter-protoplasts (Text-fig. 4, O) immediately develop cell-walls of their own, and the resulting group recalls daughter-cell formation in an *Oocystis* (Text-fig. 4, M, N, S). The daughter-cells, except for their much smaller size, are exactly like the parent. The cells thus formed do not appear to add to the bulk of the thallus, and it is not improbable that they escape after rupture of the wall of the parent-cell and serve for purposes of multiplication.

The living material kept in the laboratory was repeatedly searched for possible motile spores, but without success. The alga remained for a month in a fairly healthy condition, but after that began to deteriorate and finally died. The disintegrating and dead thalli were carefully examined for evidence of perennating spores, but none were found.

An alga resembling that just described occurred attached to the rocky bed of a stream above the water-falls at Courtallum in South India. The thalli were smaller and not nearly so abundant, but there were no other noteworthy differences.

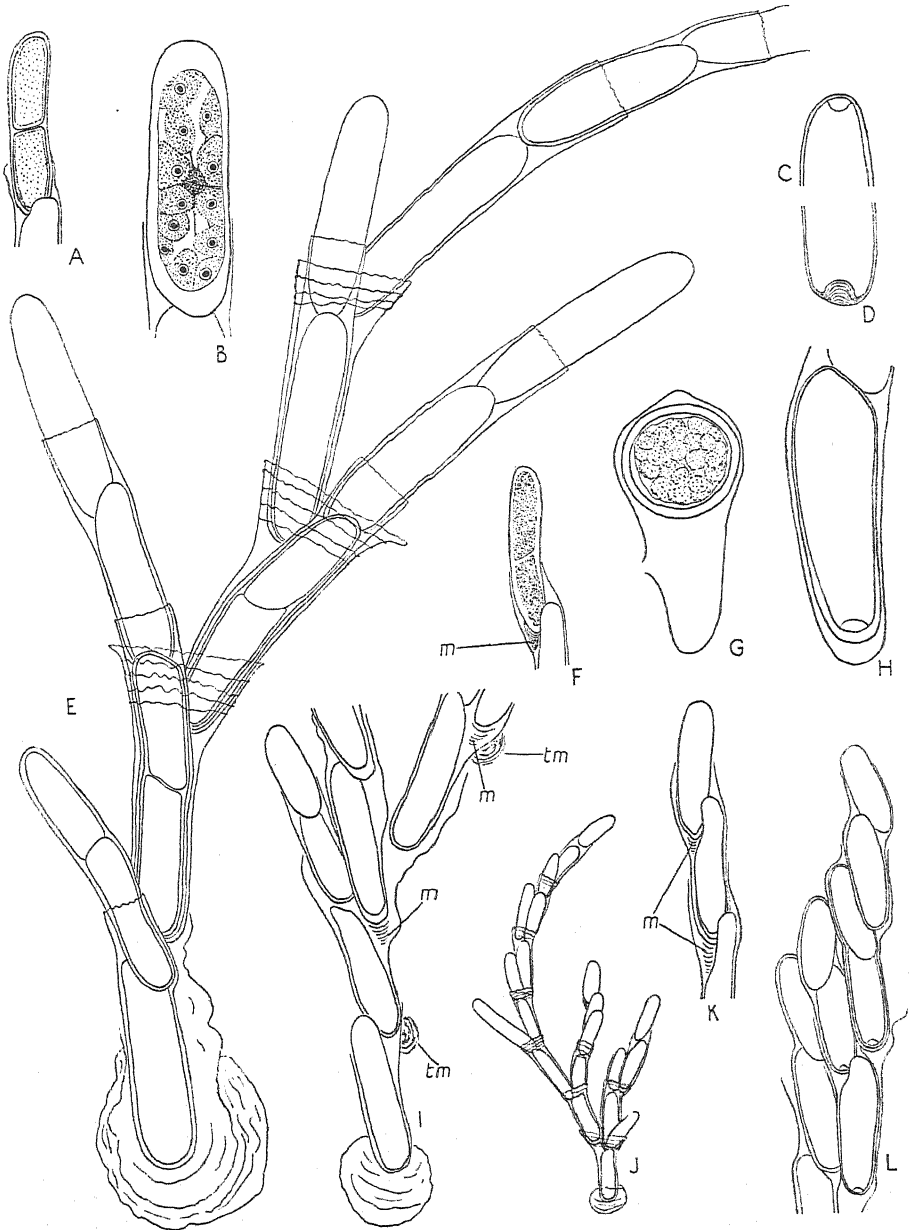
In another form (var. *pulneyensis* var. nov.), found attached to the rocky bed of a cold mountain stream at Kodaikanal in the Pulney Hills in South India, the mother-cell walls of only one generation could be detected in the mucilaginous matrix, since there was rapid gelatinization of those of the older generations (Text-fig. 4, L, P, Q). Owing to this the thallus lacked the tough consistency and the compact habit of the type, and appeared as a more expanded cushion. The cells in this form were broader in the middle than at the ends, which appeared somewhat attenuated. The pyrenoids were larger and more distinct, and at each end of the protoplast was a colourless cytoplasmic region not occupied by the chloroplasts (Text-fig. 4, I-K).

*Ecballocystis courtallensis* sp. nov. (Text-fig. 5 and Pl. VIII).

This alga<sup>1</sup> was found, along with an attached species of *Spirogyra*, on a rock which was continuously splashed by a waterfall above. It formed a thin green coating over a small area, and the material had to be scraped off carefully with the knife.

The form of the thallus is definitely dendroid (Text-fig. 5, E, J; Pl. VIII, Fig. 9). The cells are elongate and cylindrical with rounded ends, but the upper end is slightly more broadly rounded than the lower. The cell-contents are most clearly brought out with Heidenhain's iron-haematoxylin or Delafield's haematoxylin. Each cell contains a single central nucleus with a fairly prominent nucleolus and a large number (mostly 16 or 32) of disc-shaped, rounded or roughly elliptical, parietal chloroplasts, each having a minute, though definite pyrenoid (Text-fig. 5, B). The nucleus

<sup>1</sup> I am indebted to Mr. R. V. Narayanaswamy for sending me further material of this alga.



TEXT-FIG. 5. A-F, H, J, K, *Ecballocystis courtallensis* sp. nov. A, formation of two daughter-cells inside the mother-cell wall; B, single cell showing nucleus and chloroplasts; C, apical, D, H, basal nodular thickenings of the cell-wall; E, portion of a colony showing details of branching; F, division of cell-contents; J, colony showing habit; K, elongation of lower daughter-cell past the base of the upper one. G, I, L, *E. courtallensis*, f. *jogensis*. G, swollen cell with contents rounded like a cyst and having an aperture in the cell-wall (see p. 208); I, L, details of colonies; m, attaching mucilage; tm, mucilage secreted outside wall (see p. 208). Pyrenoids black. A, F  $\times 270$ ; J  $\times 115$ ; K, L  $\times 405$ ; B, C, D, G, H  $\times 800$ .

appears to be suspended in the centre of a large vacuole by means of a number of radiating cytoplasmic threads. The chloroplasts of the older cells were packed with starch, which made it difficult to see the pyrenoids in them.

The cell-wall is not coloured blue by chlor-zinc-iodide. It is thin at the top, but gradually becomes thicker towards the base of the cell. At each of the two poles there is a small nodular thickening which is only recognizable after careful examination under a high power. The thickening when examined under high magnification appears lamellated, the lamellae evidently belonging to the inner portion of the wall at the two poles of the cell (Text-fig. 5, D). This thickening is somewhat more prominently developed at the lower than at the upper end of the cell, and is conspicuous only in the older cells (Text-fig. 5, C, H, and Text-fig. 5, L for *f. jogensis*).

*Cell-division and colony-formation.* When the cell has reached a certain size the contents undergo a slightly oblique division across the middle (Text-fig. 5, F), each daughter-protoplast surrounding itself with a wall of its own (Text-fig. 5, A; Plate VIII, Fig. 10). The enlarging daughter-cells soon cause a rupture of the mother-cell wall at the top where it is thinnest and where a certain amount of gelatinization probably takes place. The upper of the two daughter-cells now projects beyond the ruptured end of the mother-cell wall, and the subsequent growth of the lower daughter-cell helps to push the upper one still further out; the lower cell, however, remains at the base of the ruptured parent-membrane. Very soon each of the daughter-cells secretes at its base a very small, more or less conical mucilage pad which seems to attach the cell with some degree of firmness to the inside of the mother-cell wall (Text-fig. 5, F, I,<sup>1</sup> K, m). As further elongation of the lower daughter-cell takes place, it pushes past the base of the attached upper cell (Text-fig. 5, K), causing a distension of the elastic mother-cell wall which is later thrown into wrinkles (Text-fig. 5, E, J).

When the daughter-cells have attained their full size a fresh division takes place, and, since the same process is repeated again and again, a richly branched colony is soon established (Pl. VIII, Fig. 9, and Text-fig. 5, E, J). Division is, however, often confined to the upper daughter-cell, the lower one either remaining undivided or dividing only once or twice. At each division one of the daughter-cells becomes attached to the base of the ruptured parent-membrane, while the other comes to be fixed just within the mouth of the latter. The whole colony is firmly attached to the substratum by a thick, approximately circular pad of tough mucilage secreted by the base of the lowermost cell of the colony (Text-fig. 5, E, J). A considerable quantity of tough mucilage is also to be found at the base of some of the oldest cells, even though they are not actually in contact

<sup>1</sup> Fig. 5, I refers to the forma *jogensis*, p. 208.

with the substratum (Text-fig. 5, 1, *tm*). The amount of mucilage in these cases is considerably greater than that on the younger cells, and it seems as though its secretion went on indefinitely. It appears, however, that contact with a firm substratum acts as a special stimulus, since the lowermost cells of few-celled colonies, and, in some cases, even the single cells of first stages, show a large mucilaginous attaching pad.

The cells of this alga display a marked degree of polarity, as exhibited by the restriction of mucilage-secretion to the lower ends, by the slightly greater breadth of the cells at the upper end, by the slightly greater thickening of the cell-wall at the base, by the invariable rupture of the wall at the upper end, and the more conspicuous development of the polar nodule at the basal pole. When single cells get dispersed from a parent-colony—at present the only known method of reproduction—contact with a substratum presumably at once induces profuse secretion of mucilage from one end of the cell, whereby attachment is effected. How the erect position of the cell is assumed is not quite clear. There is probably some kind of adjustment, as shown below in the case of *E. ramosa* (p. 215), due to localized secretion of mucilage. It is open to discussion whether the loose cells already possess polarity or whether the polarity is determined in them only after contact with the substratum is established. This can only be settled by experiment. Free cells show very slight differences in the shape of the two ends, but clear outward evidence of polarity is afforded only in the older cells.

*Reproduction.* Although numerous specimens were examined at different times, division into more than two cells was never observed. The living alga was kept under observation for some time, but no zoospores were seen. Certain empty cells which exhibit a small round hole in their wall may represent sporangia from which swimmers have escaped, but they may equally well be the result of the presence of some parasite (cf. also the structures described in *E. courtallensis* var. *jogensis*, p. 208). So far, the only certainly established method of reproduction is by the liberation of the ordinary daughter-cells from the colony.

In the character of the cells and the dendroid habit this species shows a certain resemblance to the African *E. simplex* Fritsch (cf. below, pp. 209–10), but the Indian form is composed of far more numerous cells and is profusely branched. In *E. simplex*, moreover, a considerable number of empty cell-walls are found beneath the cells and especially below the lowermost one; this feature is never found in the same degree in the Indian alga. Though numerous specimens of *E. courtallensis* collected at different times during the last seven years have been examined, none showed 'sporangia' with four or more daughter-cells, such as are very commonly found in *E. simplex* (7, Text-fig. 4, D). This indicates that in the Indian alga sporangium-formation is either lacking or of very rare occurrence.

*E. courtallensis* forma *jogensis* forma nov. (Text-fig. 5, I, L; Pl. VIII, Figs. 5, 6, 7).

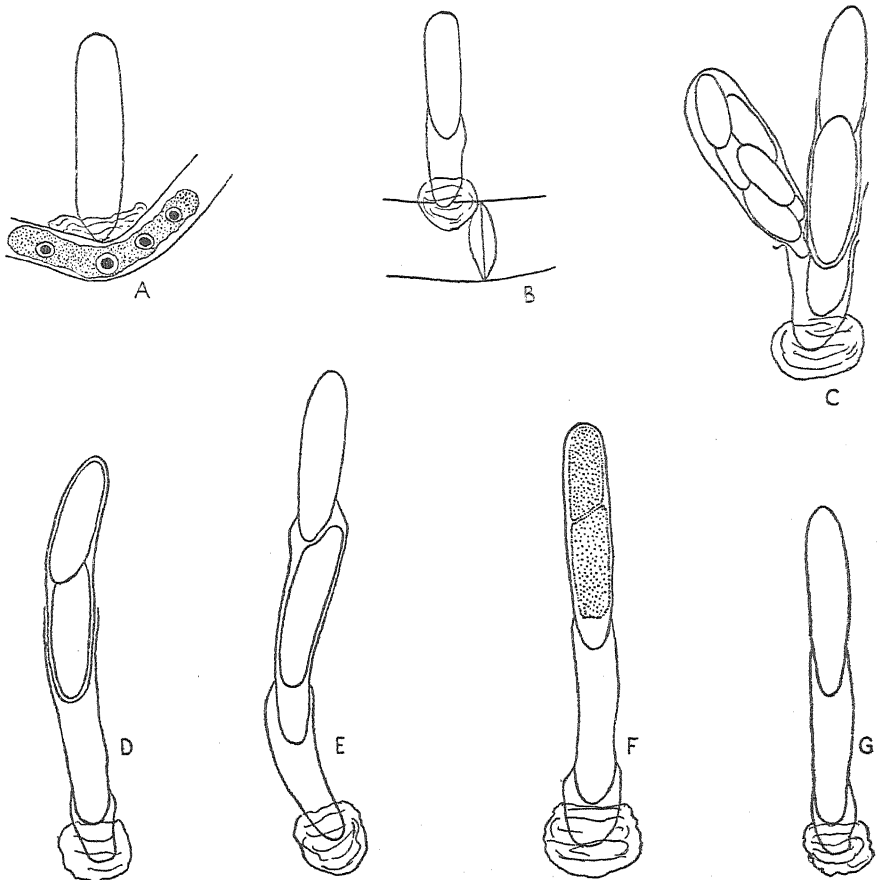
Another form of this species (f. *jogensis*) was found abundantly as a thin greenish covering on stones wetted by spray near the famous Jog Falls in the Mysore Province of South India. It is much more profusely branched than the type with more crowded branches, and the slightly smaller cells are placed nearer to each other. It may well prove to be merely a habitat-form of the main species (Text-fig. 5, I, L; Pl. VIII, Figs. 5-7.)

The difference in the habit is due to the slightly different behaviour of the daughter-cells after division, as well as to the shape assumed by the old walls of the parent cells. In this form the upper daughter-cell becomes attached *a little above the middle* of the ruptured wall of the parent-cell (Text-fig. 5, I; Pl. VIII, Fig. 7), and, when the lower cell grows past it, the original membrane becomes distended from the middle portion upwards, whereas in the type it is only the uppermost part that is affected (Text-fig. 5, E). The cell-wall, moreover, does not appear to be so elastic as in the type species, and does not show wrinkling to the same extent. The aperture remains fairly wide open (Text-fig. 5, I, L). As a result of these differences, the cells of the colony lie closer together within the widely opened mother-cell walls and the numerous branches are more closely adpressed to the 'main axis', and arise a little above the middle of the old wall, and not from near its upper end. The 'main axes' often present the appearance of consisting of two or more oblique rows of cells, which is due to the fact that the upper portion of the old mother-cell wall encloses within it the upper portion of the lower daughter-cell and the lower portion of the upper one (Text-fig. 5, L; Pl. VIII, Figs. 6, 7).

The material of this form included many loose cells in which the upper portion of the unruptured envelope was much dilated, while the contents, surrounded by a separate, relatively thick, membrane, formed a spherical mass within the swollen end (Text-fig. 5, G). Similar cells were occasionally also found within the intact thallus. The appearance is that of a sporangium containing a hypnospore serving as a possible means of perennation. Each of these cells, however, shows a definite aperture with slightly protruded edges situated on one side of the basal portion and presenting altogether the appearance of a natural opening formed by the cell itself. Since the edges of this pore are directed outwards, it would seem to be formed by gelatinization of the apex of a papilla after the manner of the formation of the pore in an oogonium of *Oedogonium*, but no such intact papillae were observed. The contents of the spore showed chloroplasts and pyrenoids and appeared quite healthy. In spite of careful examination no trace of a parasite was found, nor does the general character of the structures in question really suggest that they are due to a parasitic



organism. A very remote possibility is that we are concerned with fertilized oogonia, but their true nature can only be settled by observation of living material.



TEXT-FIG. 6. *Ecballocystis simplex*, Fritsch. A, single cell on a filament of *Mougeotia*; B, D-G, different stages of growth of the colony; C, a slightly branched colony with a sporangium containing eight daughter-cells. Pyrenoids black.  $\times 510$ .

*Ecballocystis simplex*, F. E. Fritsch (Text-fig. 6).

Through the kindness of Prof. Fritsch I have been able to examine a slide of this species, and, as a result, have come to the conclusion that the very small stature is not due to immaturity, but to the possession of a very different method of growth from that found in *E. courtallensis*. I am inclined to think that the plants figured by Fritsch may really be full-grown specimens. *E. simplex* occurs attached to water-plants and filaments of *Mougeotia* in flowing water. The plants, which often consist of but one

cell (cf. below), arise perpendicularly from the substratum, and are attached by a mucilage pad (Text-fig. 6, A-G). The cell-structure appears to be quite similar to that of *E. courtallensis*, with numerous parietal disc-shaped chloroplasts, each with a pyrenoid. In *E. simplex* it appears that of the two daughter-cells formed at any given division (Text-fig. 6, F, D), only the lower one remains as part of the colony, the upper one being gradually pushed out by the growth in length of the lower daughter-cell, as well as by a more or less pronounced upward movement of the latter inside the mother-cell wall (Text-fig. 6, E). It looks as though the mucilage secreted by the daughter-cells is neither sufficiently copious, nor tough enough, to attach them firmly to the mother-cell wall, as happens in *E. courtallensis*. The mucilage appears to be diffuent and diffuse (cf. 7, Text-fig. 4, A, F). As a consequence, when the lower cell lengthens, it simply pushes the upper one in front of it. The upward movement on the part of the lower cell may be due to the pressure of the elastic mother-cell wall which, after being distended by the enlarging daughter-cells before apical rupture takes place, will tend to shrink to its old size. This upward movement is possibly also aided by the thin mucilage secreted by the cell at its lower end. In some cases this upward movement is not considerable, but often it leads to the lower daughter-cell ultimately shifting so far up that only its conical basal portion remains within the old mother-cell wall (Text-fig. 6, B, E-G).

In this way, therefore, the upper of the two cells formed at each division will tend to become thrust completely out of the colony, so that in general only one daughter-cell will remain as part of the colony. The latter will thus undergo no great increase in size. It appears, however, that very occasionally both daughter-cells may remain adhering to the top of the mother-cell wall (cf. 7, Text-fig. 4, F, G), so that a small dendroid colony is formed. But for this occasional retention of both daughter-cells, the colony would consist of only a single cell with more or less numerous cell-membranes below it. Every division, in fact, adds another empty membrane to the group. To judge from the dimensions of the successive empty membranes, it would appear that the first cell of a colony is relatively small and that the later ones are larger. Fritsch regarded the successive ruptured membranes found at the base of the older colonies as evidence of repeated rejuvenation of the protoplast, but in the light of the experience gained from a study of the Indian species of *Ecbalocystis*, I believe that the explanation put forward above is the more probable. A study of living material of *E. simplex* would, however, alone settle the matter conclusively. In view of the fundamental difference in the *method of growth of the colony*, there can be no doubt that *E. courtallensis* is distinct from *E. simplex*, quite apart from other differences. In *E. simplex* division into 4, 8, or 16 daughter-cells is frequent.

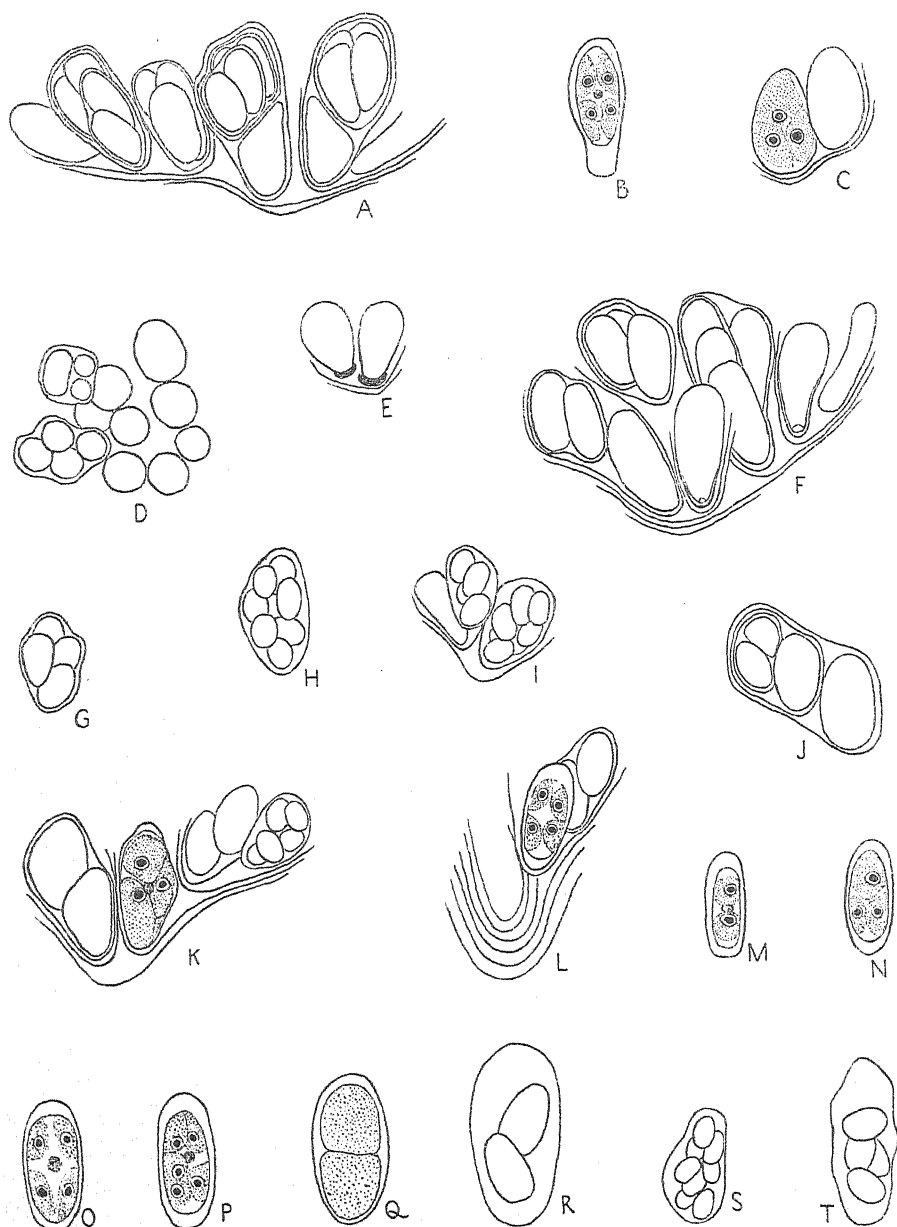
*Ecballocystis pulvinata* var. *minor* var. nov. (Text-fig. 7, A-L).

The colonies of this alga are very small with closely crowded cells, so that in the intact colony only the rounded ends of the cells seen in vertical view are presented to the observer (Text-fig. 7, D). If a colony is subjected to gentle pressure under a cover-glass, the cells are seen spread out, and if previously stained for a few minutes with Delafield's haematoxylin, Congo red, or methylene blue, the dendroid nature of the alga becomes clear.

The general arrangement of the cells is very similar to that shown in Bohlin's figure of *E. pulvinata* (2, Tab. I, Figs. 1-4), since the thallus as a whole is broadly obconical, and spreads obliquely over the substratum (Text-fig. 7, A, F, K). The conical gelatinous processes at the bases of the cells recorded by Bohlin are, however, replaced by a thin layer of mucilage which serves to attach the cells to the mother-cell wall (Text-fig. 7, E). The old mother-walls undergo gelatinization to some extent and help to attach the thallus to the substratum.

The cells are oblong to irregularly elliptic in shape, but they are often somewhat narrowed towards their lower end and then appear sub-conical with a truncate base and a broadly rounded apex (Text-fig. 7, B). Bohlin was unable to give any details as to the cell-contents in his species, but in the present form, by careful staining with Delafield's haematoxylin, a central nucleus with four parietal disc-shaped chloroplasts, each with a pyrenoid, can be more or less clearly seen (Text-fig. 7, B, C, K, L). The arrangement of nucleus and chloroplasts is similar to that seen in a cell of *E. Fritschii* containing four chloroplasts. In some cells the wall shows a slight polar thickening, rather similar to, but not so distinct as, that found in *E. courtallensis*; when present, this thickening is more pronounced at the lower than at the upper pole (Text-fig. 7, F). Division of the cells takes place in the usual way, but the resulting daughter-cells do not alter their respective positions to any considerable extent subsequent to division, and are, in later stages, found standing side by side at the base of the membrane of the parent-cell, the lower daughter-cell only very slightly below the upper one (Text-fig. 7, C, E, F, J). The upper one is generally situated on the side of the mother-cell directed towards the periphery of the colony, while the lower one is located towards the inside. Further division is usually confined to the upper daughter-cell and, as a result of this and of the fact that this cell is usually placed on the outside, the spreading habit of the colonies is brought about. Moreover, since the daughter-cells remain near the base of the parent-cell, the whole colony is procumbent and does not possess the erect branched habit of the previously described species of *Ecballocystis*.

As in the other cases, the old mother-cell walls of successive generations



TEXT-FIG. 7. A-L, *Ecballocystis pulvinata*, Bohlin var. *minor*, var. nov. A, C, F, K, L, portions of colonies; B, single cell with chloroplasts and nucleus; D, vertical view of cells of a colony; E, two cells showing basal mucilaginous secretion; G, H, I, sporangia with four or eight daughter-cells; J, several cell-generations included in the respective mother-cell walls; M-T, *Oocystis ecballocystiformis*, sp. nov. M-P, cells showing contents; Q, division of the protoplast; R, S, T, formation of autospores. Pyrenoids black. All  $\times 890$ .

persist for some time and are to be seen in considerable numbers as somewhat swollen distinct lamellae below the cells.

The cell-contents very often divide into four or eight daughter-cells (Text-fig. 7, G-I, K), and any cell of the colony may develop into such a 'sporangium'. Bohlin did not encounter such stages in his material. On the other hand, it is not uncommon to find a number of cell-generations enclosed in the unruptured membrane of the parent (Text-fig. 7, J). This is also shown in Bohlin's figure of the type (2, Fig. 4).

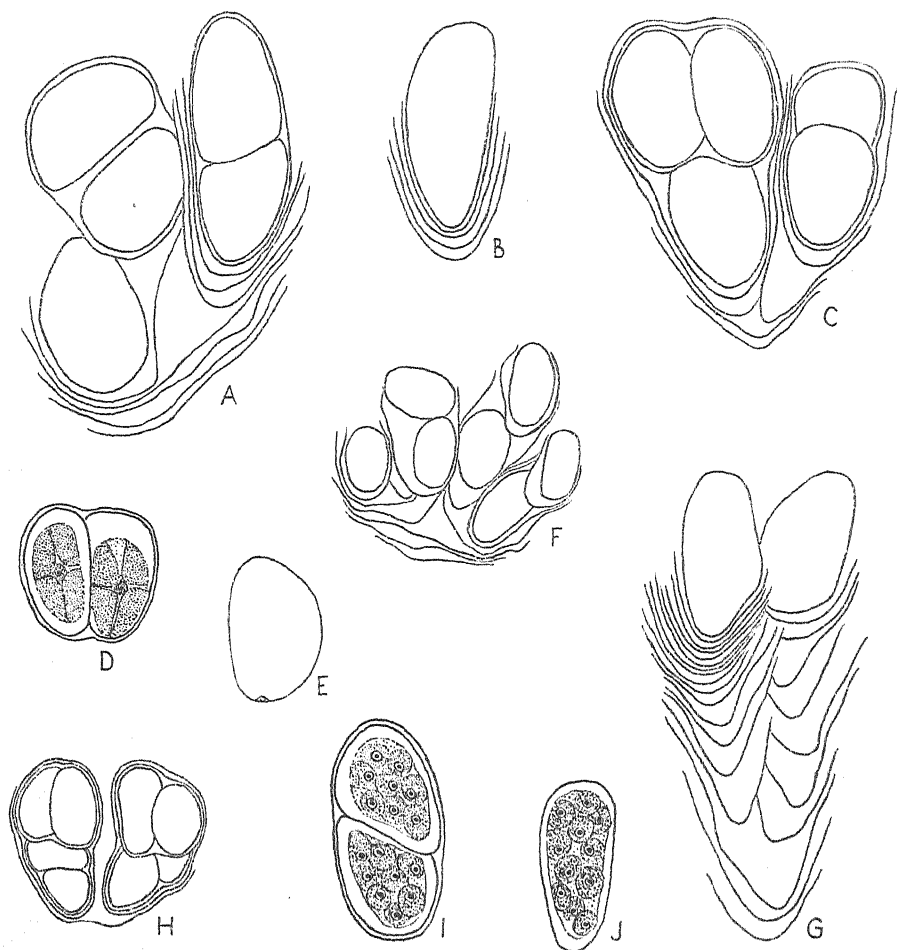
*Ecballocystis pulvinata* Bohlin var. *diffluens*, var. nov. (Text-fig. 8).

This alga formed a minute cushion-like colony about 0.5-1.5 mm. in diameter, and was found growing along with *E. courtallensis* f. *jogensis* on stones constantly wetted by spray from the Jog Falls. It shows some resemblance to *E. pulvinata* var. *minor* in its general appearance. The colony consists of numerous short, more or less erect, dendroid branches closely aggregated together. Owing to the dense growth, the structure is not easily made out, but if a colony is gently squeezed under a cover-glass, the erect branches are spread out, and some of them may be seen very clearly at the edges where they happen to lie clear of the others. The base of the whole colony is obconical or broad (Text-fig. 8, A, C, F-H). The arrangement of the cells is somewhat similar to that of *E. pulvinata* var. *minor*, but there are marked differences in the shape and size of the cells, as well as in the structure of the cell-contents. The cells are broadly and often irregularly elliptic, sometimes narrowed at the base and broadly rounded above. They possess a central nucleus and 4, 8, or 16 parietal disc-shaped chloroplasts each with a small pyrenoid (Text-fig. 8, D, I, J). In some of the fully-grown cells a very minute nodular thickening of the wall can occasionally be seen at the base (Text-fig. 8, E), although never discernible at the upper pole.

The walls of the older generations undergo gelatinization relatively quickly, with the result that the thallus is more diffuse than in var. *minor*. Moreover these walls are thin and delicate, and only to be detected with difficulty, so that the thallus, unless carefully stained, appears as a structureless mass of cells, and its dendroid nature is not at first apparent. Cell-division takes place as in *E. pulvinata* var. *minor*, but only two daughter-cells are formed. Division into four or more daughter-cells has not been observed, but as in var. *minor*, two or three cell-generations are often found enclosed in the unruptured parent membrane (Text-fig. 8, H). In the upper portions of the dendroid branches, one of the daughter-cells is often seen to have escaped, probably serving for purposes of propagation (Text-fig. 8, F). It is not unusual to find single cells with numerous old walls below them (Text-fig. 8, B, G) at the ends of some of the branches

indicating that, as in *E. simplex*, repeated cell-division has occurred, accompanied by liberation of the upper daughter-cell.

The alga just described differs from *E. pulvinata* var. *minor* in the



TEXT-FIG. 8. *Ecbalocystis pulvinata*, Bohlin, var. *diffluens*, var. nov. A, C, F, H, portions of colonies; B, cell with many old membranes below it; D, I, J, cells with contents (in I showing cell-division); E, cell with nodular thickening; G, portion of colony with two cells having numerous old membranes below each. Pyrenoids black. All  $\times 950$ .

larger size and different shape of its cells, in forming only two daughter-cells at each division, and in the presence of 4, 8, or 16 chloroplasts in the cells, whereas var. *minor* never has more than eight. It agrees with *E. pulvinata* Bohlin in forming only two daughter-cells during cell-division, but differs from it in several other respects.

*Ecballocystis ramosa* Fritsch (Text-fig. 9).

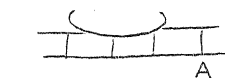
This African species was found by Fritsch<sup>1</sup> epiphytic upon attached filaments of *Monogotia*, *Ulothrix oscillarina*, and *Hormidium subtile* in flowing water. Compared to the other species of the genus, *E. ramosa* has a very simple thallus. The colony begins as a single erect cell attached by a well-developed mucilage pad. This cell usually gives rise to four daughter-cells (Text-fig. 9, L) which, as they increase in size, distend the mother-cell wall so that it finally ruptures at the apex (Text-fig. 9, M, P). One daughter-cell usually stays at the base of the ruptured membrane, while it appears that one of the remaining three is generally pushed out of the parent-membrane. The other two become attached to the aperture of the ruptured wall by means of mucilage, well seen in stained preparations (Text-fig. 9, R). Sometimes three daughter-cells are found attached at the aperture (Text-fig. 9, N). In other cases division into eight occurs (Text-fig. 9, T). In such cases, one daughter-cell seems to remain at the base of the ruptured parent-membrane, a few are extruded and the remainder become attached at the aperture (Text-fig. 9, U, W). Division into two is not uncommon (Text-fig. 9, O) and, in that case, one cell may remain at the base, while the other either becomes attached to the aperture of the ruptured wall (Text-fig. 9, V) or escapes to the outside; just as commonly, however, both daughter-cells are found attached to the aperture (Text-fig. 9, Q).

The daughter-cells divide again to form a small dendroid colony, with two or three generations of cells, though not so compact as in most other species of the genus (Text-fig. 9, S). The whole remains quite small, possibly owing to the temporary nature of the substratum.

Multiplication appears to be effected by means of the daughter-cells, which are set free and float away. These cells are often somewhat conical at one end and broadly rounded at the other, i.e., they exhibit a clear polarity before attachment to the substratum is effected. They are always found attached by the conical ends. Such cells commonly exhibit a slanting position upon the threads of the algae which serve as substratum; some lie very nearly parallel to them (Text-fig. 9, B). In the former case most of the basal mucilage is found to be secreted on the side of the cell away from the substratum, and very little on the opposite side. In cells exhibiting a more erect position a larger quantity of mucilage is found on the side adjacent to the substratum, while in the fully erect specimens the amount of mucilage is equal on both sides. All transitions are to be found from a very acute angle, with the cell almost parallel to the substratum, to a definitely erect position.

These various stages (Text-fig. 9, B-G) suggest that, when such loose

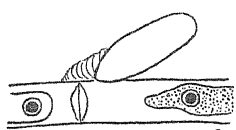
<sup>1</sup> Prof. Fritsch very kindly allowed me to examine some of his material of *E. ramosa* from S. Africa.



A



B



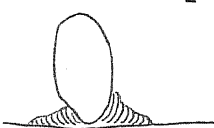
C



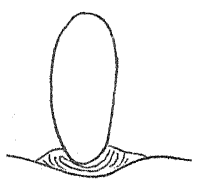
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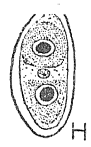
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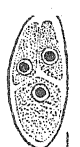
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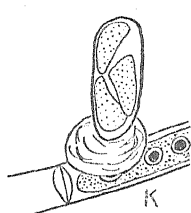
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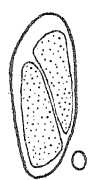
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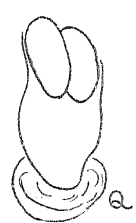
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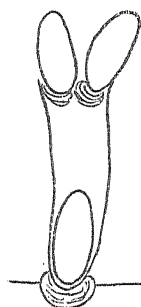
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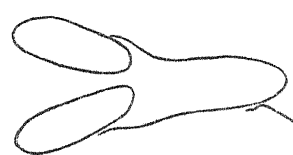
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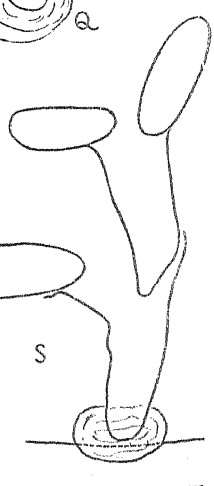
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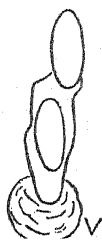
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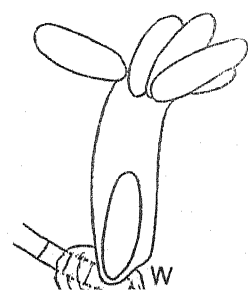
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cells happen to be floated on to a suitable filament, the stimulus of contact induces a secretion of attaching mucilage from the conical pole which at first ensues on the outer side only. Later secretion of mucilage also takes place on the side adjacent to the substratum, and the cell gradually assumes an erect position. Single loose cells were often found lying flat on the algal filaments, a position which might be due to the accident of mounting (Text-fig. 9, A). In view of the many instances of oblique attachment, however, in which in every case there was a definite secretion of mucilage from the basal pole, these cases may possibly represent the first stages in the establishment of the alga upon the filaments.

These observations on *E. ramosa* may indicate the manner in which the cells of the other species of *Ecballocystis* become attached to and erected upon the substratum.

The contents of the cells of this species resemble those of *E. Fritschii*. There is a central nucleus and 2, 4, or 8 parietal disc-shaped chloroplasts each with a pyrenoid (Text-fig. 9 H, I, J). The chloroplasts are very delicate and lie closely crowded, so that they are not very clearly defined.

#### *The Affinities of Ecballocystis.*

The systematic position of *Ecballocystis* is very uncertain. Bohlin (2), when he first established the genus, placed it among the Tetrasporaceae, and suggested that it was nearly related to the Flagellata, and especially to the genera *Euglenopsis* (6), *Chlorangium* (15) and *Prasinocladus* (9). He considered that it stood nearer to the two last-named genera, in which the cells are attached by broad mucilaginous stalks secreted at their lower ends, and suggested that *Ecballocystis* might be regarded as a *Chlorangium* in which the mucilaginous stalks remained undeveloped.

Wille (20, pp. 27, 28) likewise referred it to Tetrasporaceae (tribe Hauckieae), including in it Setchell and Gardner's *Collinsiella*; West (17, pp. 185-189) and Printz (19, p. 69), on the other hand, place it in the tribe Chlorangiaceae of the Tetrasporaceae, and retain *Collinsiella* as a distinct genus in the tribe Palmophylleae. Oltmanns (12, p. 138) includes *Ecballocystis*, together with *Chlorodendron* and *Prasinocladus*, in the new family Chlorodendraceae of Volvocales (cf. also 12a, p. 240). Lemmermann (10, p. 25) places it in the family Chlorangiaceae of the group Tetrasporales. Fritsch (7, pp. 494-502), in describing the species *E. ramosa* and *E. simplex*, lists the genus under Chlorodendrales, which is ranked as a group of equal status to the Tetrasporales and Chlamydomonadales, the three

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TEXT-FIG. 9. *Ecballocystis ramosa*, Fritsch. A-C, stages in the attachment of cells to the substratum; H-K, cells with contents (in K showing division); L, formation of four daughter-cells; M, N, P, R, different types of attachment of daughter-cells to the ruptured mother-cell wall; Q, V, the same in a cell with two daughter-cells; T, U, W, the same in a cell with eight daughter-cells; O, division of protoplast into two; S, loose dendroid colony. Pyrenoids black. All  $\times 560$ .

being regarded as representing different evolutionary lines of the Volvocales (cf. also 18, p. 67). He regards *Ecballocystis* as quite distinct from *Collinsiella*. Printz (13, pp. 78, 79) very rightly refers the three species, described by Yendo (21) under *Ecballocystis*, to *Collinsiella*.

All authorities are thus agreed in placing *Ecballocystis* among the simpler Chlorophyceae, but while some regard it and the related genera merely as a tribe of the Tetrasporaceae, Oltmanns and Fritsch include them in a group with a higher status, the latter even regarding the Chlorodendrales as a distinct evolutionary line (cf. also 8, p. 109). The latter authority, moreover, considers the genera grouped in Chlorodendrales to be closely related to the motile forms from which they probably originated. Of the divers botanists who have dealt with the systematic position of the genus, only Bohlin and Fritsch actually investigated it, and both had scanty preserved material at their disposal in which the details of cell-structure were not easily decipherable. The possession of chloroplasts with pyrenoids, and the presence of starch in the cells of the forms here described fully confirms the reference to Chlorophyceae.

As regards the relation of *Ecballocystis* to the other genera of Chlorodendraceae, the writer's investigations have shown that in its possession of several or many parietal chloroplasts, each with a pyrenoid, it diverges markedly from the others. While there is a general resemblance embodied in the dendroid habit, the detailed manner of colony-formation is not the same. And in the third place the apparent absence of motile reproductive stages, considered more fully below, removes *Ecballocystis* from the other three genera with which it has usually been associated. In view of the rather decided differences *Ecballocystis* would perhaps best be placed by itself in a separate family of Chlorodendrales. In the writer's opinion its nearest allies are to be sought elsewhere, as will be explained in the following.

Emphasis has just been laid on the non-discovery of spores. Fritsch (7) searched for zoospores in his material, but in every case the loose cells found in the sediment were clothed with a wall. He suggested that this might be due to any zoospores present having formed a wall during the interval between collection and preservation of the material. It has been possible to study the Indian members of the genus in a living condition, both in nature and in the laboratory, on *many different* occasions, but no motile stages have ever been observed. There is thus reason to suspect the absence of motile spores. Such negative evidence is not, however, sufficient to dismiss the possibility of an occasional formation of swarmers, the more so as many empty cells from which the contents had apparently escaped were often to be found in the colonies.

In the absence of swarmers attachment to the substratum is not easily visualized. In flowing water, or in the littoral zone of lakes where the water is agitated, the cells of Zygnemaceae, however, become attached

without possessing motility. And the same may be true of *Ecballocystis*. In either case a ready secretion of attaching mucilage must take place. Assumption of the erect position must depend upon some erecting sense, and is evidently brought about by a localized secretion of mucilage (cf. the data above given for *E. ramosa*, p. 215).

During cell-division in *Ecballocystis* the contents divide obliquely into two, and very soon new membranes are formed around the daughter-protoplasts, which, by further growth, ultimately rupture the wall of the parent-cell, usually at its upper end. This is seen in Bohlin's type-species, in the two species described by Fritsch, as well as in all the species collected by me. In *E. ramosa*, Fritsch, *E. simplex*, Fritsch, *E. Fritschii* and *E. Fritschii* f. *pulneyensis*, the cell-contents occasionally divide into more than two daughter-cells (four, eight, or sixteen) without the rupture of the mother-cell wall. And in all such cases the daughter-protoplasts surround themselves with their own individual cell-walls soon after their production. Such a formation of daughter-cells recalls to a very marked extent an *Oocystis* forming 'autospores' inside it, although oblique division is not recorded in this genus. The daughter-cells in *Ecballocystis*, even when there are only two, may in fact be regarded as *autospores*. The formation of 2, 4, 8, or 16 autospores in the cells of different species of *Ecballocystis* is fully paralleled among the species of *Oocystis*. The ordinary cell-multiplication of other Chlorodendraceae is likewise of the nature of 'autospore formation', although none of the other genera except *Chlorangium* are recorded as showing division into more than two daughter-cells.

The cell-contents in the Indian species of *Ecballocystis* are very similar to those of certain species of *Oocystis*. Thus in a species of *Oocystis* (which comes near to *O. crassa*, Wittrock), collected from an artificial tank in the University Botanical Garden at Heidelberg last September, the cells contain a central nucleus and a number (2, 4, or 8) of parietal disc-shaped chloroplasts with a single pyrenoid. The same arrangement of the chloroplasts is found in *O. Borgei*, Snow and *O. lacustris*, Chodat (4, Pl. XXII, Figs. 4, 8, 9, and 13). *O. eremosphaeria*, Smith (14, p. 113, Pl. XXIII, Figs. 1 and 2) has numerous chloroplasts with a pyrenoid in each. In another species of *Oocystis* collected near the Jog Falls some years ago, the resemblance is so close that the cells might readily be mistaken for the free cells of *E. Fritschii*. The species in question may be named

*Oocystis ecballocystiformis* sp. nov. (Text-fig. 7, M-T).

This species occurred as a light-green floating scum on the surface of the water occupying a depression in the rock, about thirty yards from the spot where *E. courtallensis* f. *jogensis* and *E. prostrata* were found. This scum consisted of numerous free-floating cells which were in all respects (including size) exactly like the loose cells of *E. Fritschii*. They were

oblong-elliptic in shape, with a central nucleus and 2, 4, or 8 parietal disc-shaped chloroplasts, each with a pyrenoid (Text-fig. 7, M-P). There was no difference in the thickness of the cell-wall at the two ends. The cell-division was not oblique, but nearly transverse, as in *Oocystis* (Text-fig. 7, Q). Occasionally two, four, or eight daughter-cells were found loosely enclosed within the much distended mother-cell wall (Text-fig. 7, R-T). The daughter-cells evidently become free by the dissolution of the latter, but actual instances of this were not available in the material.

It is possible that these are actually loose cells of *E. Fritschii*, and that this is the form in which this alga reproduces. On the other hand, the normal *E. Fritschii* has not been met with in the neighbourhood of the Jog Falls, its nearest recorded localities being more than two hundred miles away. In view of this fact and of the other distinctive features, viz., absence of polarity, transverse division, and the occurrence of cells lying loosely within the distended mother-cell wall, it would seem best for the present to regard it as a species of *Oocystis*, with cells very similar to those of *Ecballocystis*.

The large number of chloroplasts found in the dendroid species of *Ecballocystis* is no doubt due to the larger size of the cells.

In some of the species of *Ecballocystis* here described the wall is often somewhat thickened at the lower end, although this thickening is often seen only in the older cells. Definite polar thickenings, like those of many species of *Oocystis*, are only seen in *E. courtallensis*. The presence of such polar thickenings in this genus may be of significance, since even in *Oocystis* there are quite a number of species which lack this feature. In two important respects, therefore, in the mode of formation of daughter-cells and in the structure of the cells, there are considerable resemblances between *Ecballocystis* and certain species of *Oocystis*.

On the other hand, there are marked points of difference, such as the constant oblique division in *Ecballocystis* and the pronounced difference between base and apex in the cells. This finds its expression in the greater thickening of the membrane in the basal part,<sup>1</sup> the almost invariable rupture of the membrane in the apical part, and the frequent secretion of special mucilage pads only from the lower ends of the cells.

In connexion with my investigations on *Ecballocystis Fritschii* it was observed that, as the hill stream dwindled with the onset of the dry season, the plants in the tiny pools of water remaining at the sides became less compact and more diffuent, as if they were undergoing gradual dissolution.

<sup>1</sup> One species of *Oocystis*, *O. apiculata*, W. West, shows a certain amount of polarity, the cells having only one polar thickening (cf. 16, p. 125, and Fig. 97).

There were plenty of loose cells in the material, though they did not present an unhealthy appearance. Evidently the conditions in the stagnant water were not favourable for a dendroid growth, and the colonies were breaking up into the individual cells.

If such cells became adapted to life in stagnant water, and lost the faculty of dendroid growth, though still retaining the same method of daughter-cell formation (2, 4, 8, or 16 'autospores' within the mother-cell wall), there would be a close approach to *Oocystis*. Under such new conditions of life the cells might be expected ultimately to lose their polarity, and would be indistinguishable from an *Oocystis*. Though most species of *Oocystis* are non-colonial, *O. gloeocystiformis*, Borge, shows the retention of several generations of daughter-cells within the membranes of successive generations. Similarly, in the closely allied genus *Nephrocytium*, *N. ecdysiscepanum*, W. & G. S. West forms a diffuse colony in which 'several generations are aggregated in a fan-shaped manner, owing to ecdysis, but incomplete dissolution of the old mother-cell walls' (17, p. 197). This latter species recalls *E. Fritschii* in the arrangement of the old mother-cell walls (cf. 18, Fig. 36, B). It is not impossible that the two algae just mentioned represent transitional stages between an *Ecballocystis* (dendroid) condition and an *Oocystis* (free, unicellular) condition.

On the other hand, the dendroid *Ecballocystis* condition might be derived from unicellular forms like *Oocystis* as an adaptation to life in rapidly flowing water. If an *Oocystis* acquired polarity, and developed the capacity to secrete mucilage at one end of the cell, whereby attachment to a substratum became possible, and, if the daughter-cells, after the rupture of the mother-cell wall, secreted mucus in the same way and remained attached to the latter, a dendroid colony like that of *E. ramosa* or *E. courtallensis* would result. While, if all the old walls underwent gelatinization, a form like *E. Fritschii* would be obtained. Forms like *Oocystis gloeocystiformis* and *Nephrocytium ecdysiscepanum* would fit well into such a scheme.

This second alternative does not, however, appear likely for the following reasons. Polarity is probably an earlier condition associated with the presumably primitive motile habit. Whatever the mode of propagation of *Ecballocystis* may be at the present day, it is extremely probable that it originated from a motile form which settled down by attaching itself to some substratum by the anterior end. Of two forms, one with and one without polarity, the former may be considered as standing nearer to the motile ancestral type. And it is easier to conceive of the loss of an already existing polarity than the acquisition of polarity by a form devoid of it. The derivation of an *Ecballocystis* with its pronounced polarity from *Oocystis* therefore seems very improbable. The oblique division of the protoplast seen in *Ecballocystis* marks a more primitive condition than the transverse one of *Oocystis*.

## DIAGNOSIS OF THE NEW FORMS.

*Ecballocystis pulvinata* var. *minor* var. nov.

Thallus forming a minute procumbent expanse on the substratum ; cells densely crowded (the ends alone visible in the intact colony), oblong to irregularly elliptical, each with a central nucleus and four parietal chloroplasts containing a minute pyrenoid, cell-wall occasionally with polar thickenings visible only under very high magnifications, that at the lower pole more marked than that at the upper ; cell-division oblique, the two daughter-cells subsequently standing nearly erect and side by side at the base of the membrane of the parent-cell, the lower daughter-cell being only very slightly below the upper one ; further division usually confined to the upper cell, so that successive divisions lead not to an erect branched growth, but to an horizontal, somewhat procumbent, broadly obconical thallus ; successive mother-cell walls undergoing slight gelatinization and found in considerable numbers as somewhat swollen distinct lamellae at the base of the younger cells ; secretion of mucus confined to a small mass at the base of each cell ; division into four and eight very common.

Dimensions of the fully grown cells:  $14 \times 8.75 \mu$ ,  $15 \times 9 \mu$ ,  $16 \times 10 \mu$ ,  $18 \times 8.5 \mu$ ,  $12.25 \times 8.5 \mu$ ,  $11.5 \times 7 \mu$ .

*Hab.* Growing along with *E. courtallensis* on a rock continuously splashed by the Shenbagadevi Falls at Courtallum, South India.

*Ecballocystis pulvinata*, Bohlin, var. *diffluens* var. nov.

Thallus forming minute diffluent cushions on the substratum, in which the densely crowded cells can be seen arranged in numerous dendroid systems growing closely together side by side. Cells elliptic-oblong to broadly or irregularly elliptic, often narrowed at the base and broadly rounded above ; each with a single nucleus and 4, 8, or 16 closely packed parietal disc-shaped chloroplasts with a single pyrenoid. Cell-division into two daughter-cells only observed ; walls of older generations gelatinizing rapidly and hard to distinguish ; sporangia with four or more cells not observed.

Dimensions of cells:  $19 \times 12 \mu$ ,  $18.8 \times 12.8 \mu$ ,  $19 \times 11.8 \mu$ ,  $20 \times 14 \mu$ ,  $21 \times 12 \mu$ ,  $21 \times 14 \mu$ ,  $21 \times 15 \mu$ ,  $22.5 \times 13 \mu$ ,  $23 \times 9 \mu$ ,  $24.5 \times 14 \mu$ .

*Hab.* On stones wetted continuously by spray from the Jog Falls in Mysore Province, South India, growing together with *Ecballocystis courtallensis* f. *jogensis*.

*Ecballocystis Fritschii*, sp. nov.

Thallus macroscopic, irregularly rounded, lobed, sometimes somewhat cylindrical, the two-daughter-cells usually formed at each division both

passing to the mouth of the ruptured parent-membrane, with the result that in the older thalli nearly all the living cells are to be found at the periphery, while the inner part consists mostly of mucilage and empty cell-membranes; cells devoid of conical gelatinous processes at their lower ends, the older mother-cell walls conical or funnel-shaped at the base; cells  $16-25\ \mu$  long and  $6-11\ \mu$  broad. Cells occasionally dividing into four daughter-protoplasts, which clothe themselves with cell-walls while still inside the mother-cell wall, and possibly serve for reproduction.

Dimension of cells:  $16 \times 7.5\ \mu$ ,  $17 \times 8\ \mu$ ,  $17.5 \times 6\ \mu$ ,  $17.5 \times 7\ \mu$ ,  $18 \times 7.5\ \mu$ ,  $19 \times 8.5\ \mu$ ,  $20 \times 9\ \mu$ ,  $22 \times 11\ \mu$ ,  $29 \times 9\ \mu$ . Newly formed cells inside the mother-cell wall:  $12 \times 5.75\ \mu$ ,  $13.75 \times 5.5\ \mu$ .

*Hab.* Growing attached in crowded masses to the sloping rocky bed of a small mountain stream just above a waterfall, Mamandur, South India. February, 1930.

*Ecballocystis Fritschii* var. *pulneyensis* var. nov.

Thallus macroscopic, forming minute, spreading, approximately round or elliptic-oblong gelatinous cushions, less compact than those of the type, about one-quarter to half an inch broad and about one-eighth to one-sixth of an inch in thickness; mother-cell walls of only one generation distinguishable, owing to rapid gelatinization of the older walls; cells broader in the middle than at the somewhat attenuated and occasionally sub-truncate ends; chloroplasts similar to the type in number and arrangement, but not so sharply marked off from one another; pyrenoid larger and distinct, with a clear starch-sheath; protoplast with a colourless cytoplasmic area at each end. Cells usually dividing into two, occasionally into four.

Dimensions of cells:  $17 \times 8\ \mu$ ,  $16 \times 5.75\ \mu$ ,  $15 \times 6\ \mu$ ,  $17 \times 7.5\ \mu$ ,  $19 \times 7\ \mu$ ,  $18 \times 7.25\ \mu$ ,  $14.5 \times 7.8\ \mu$ ,  $17 \times 7.25\ \mu$ ; the width being that of the broadest part of the cell.

*Hab.* Attached to the rocky bed of a cold mountain stream with a fairly rapid current at Kodaikanal in the Pulney Hills in South India. The depth of the flowing water was about six inches.

*Ecballocystis courtallensis*, sp. nov.

Thallus microscopic, forming a dendroid, many-celled colony, consisting of a false axis with a number of short laterals, the whole attached to the substratum by a prominent mucilaginous pad secreted at the lower end of the basal cell; the two daughter-individuals formed in cell-division become attached to the mother-cell wall by a basal secretion of mucus, the lower one near the base, the upper one near the aperture; subsequent division often confined to the upper daughter-cell, although the lower one may divide once or twice and give rise to a false branch. Cells elongate-cylindrical with

rounded ends,  $9.5-16 \times 37-84 \mu$ , the upper end slightly broader than the lower, with a central nucleus and many rounded or sub-elliptic disc-shaped parietal chloroplasts, each with a small pyrenoid; cell-wall slightly thicker at the base than elsewhere, with nodular thickenings at either pole conspicuous in the older cells. Reproduction apparently only by cells detached from the colonies; no swarmers observed.

Dimensions of cells;  $11 \times 63 \mu$ ,  $12 \times 47 \mu$ ,  $13 \times 53 \mu$ ,  $14 \times 57 \mu$ ,  $14 \times 59 \mu$ ,  $14 \times 66 \mu$ ,  $14 \times 84 \mu$ ,  $16 \times 70 \mu$ .

*Hab.* On a rock splashed by water below the Shenbagadevi Falls at Courtallum, Tinnevely District, South India; first collected in October, 1924.

*Ecballocystis courtallensis*, forma *jogensis*, forma nov.

Thallus very similar to that of the type, but with more profuse and more closely adpressed branches, the upper daughter-cell after every division becoming attached *a little above the middle* of the mother-cell wall which, as a result of the further enlargement of the lower cells, becomes distended from the middle upwards, and not only at the very top as in the type. Cells slightly smaller.

Dimensions of cells:  $10.5 \times 36.75 \mu$ ,  $10.5 \times 31.5 \mu$ ,  $10.5 \times 45.5 \mu$ ,  $11.5 \times 47.25 \mu$ ,  $12.25 \times 49 \mu$ ,  $11.5 \times 59.5 \mu$ .

*Hab.* On stones constantly wetted by spray from the Jog Falls, Mysore Province, South India.

*Oocystis ecballocystiformis*, sp. nov.

Cells oblong-elliptic, with broadly rounded ends; cell-wall thin and devoid of polar thickenings; chloroplasts 2, 4, or 8, parietal, disc-shaped, each with a minute pyrenoid; 2, 4, or 8 autospores formed inside the distended mother-cell wall.

Dimensions of cells:  $18 \times 7 \mu$ ,  $19.6 \times 8.9 \mu$ ,  $19.75 \times 8.75 \mu$ ,  $24 \times 9.1 \mu$ ,  $20.5 \times 9 \mu$ ; young cell,  $16 \times 5.5 \mu$ .

*Hab.* In a small pool occupying a depression in a rock near the Jog Falls in Mysore Province, South India.

*Artificial Key to the Species of Ecballocystis.*

- A. Colony with free branched axes not embedded in mucilage, often microscopic.
  - I. Colony composed of few cells only, microscopic, one or more of the daughter-cells formed in division commonly thrust out of the colony.
    - (a) Colony a small dendroid branch system, cells relatively short, not exceeding  $48 \mu$  in length, with 2-8 somewhat angular disc-



shaped chloroplasts whose limits are indistinct; cell-division usually into four, occasionally into two or eight . . . . *E. ramosa*.

(b) Colony commonly composed of a single cell surmounting a number of empty membranes, cells longer, up to  $67\mu$  in length, cylindrical with many disc-shaped distinct chloroplasts (up to 32); cell-division usually into two . . . . *E. simplex*.

II. Colony well-branched, composed of numerous cells; cell-division only into two, both daughter-cells usually remaining attached to the mother-cell wall . . . . *E. courtallensis*.

(i) Upper daughter-cell attached near the ruptured apex of the mother-cell wall, often bending outwards . . . . *f. typica*.

(ii) Upper daughter-cell attached a little above the middle of the mother-cell wall, nearly parallel to the lower . . . . *f. jogensis*.

B. Colony relatively large, appearing as a lobed or cushion-shaped gelatinous mass, macroscopic, with closely crowded branched axes, sometimes embedded in mucilage.

I. Colony lobed, cells embedded in the mucilage formed by the gelatinization of the old walls; daughter-cells attached to the mouth of the funnel-shaped mother-cell wall . . . . *E. Fritschii*.

(i) Colony relatively compact, cells elliptic-oblong, not broadened in the middle; walls of several generations visible . . . . *var. typica*.

(ii) Colony somewhat diffuse, cells elliptic, broadened in the middle; walls of only one generation visible, the others gelatinizing very quickly . . . . *var. pulneyensis*.

II. Colony minute, cushion-shaped, with fairly firm mother-cell wall, not gelatinizing quickly, daughter-cells not attached to the mouth of the obconical or widely cup-shaped mother-cell; old mother-cell walls of many generations lying close together *E. pulvinata*, Bohlin.

(i) Daughter-cells attached by conical gelatinous processes secreted at their lower end; cell-divisions only into two; chloroplasts unknown . . . . *var. typica*.

(ii) Daughter-cells attached by a broad gelatinous secretion from their lower end; cell-division into two to eight daughter-cells; chloroplasts four or eight, in the form of angular discs *var. minor*.

(iii) Daughter-cells not attached by any definite secretion; walls delicate and rapidly gelatinizing; cells larger and more rounded, irregularly elliptical; chloroplasts many, up to sixteen, in the form of rounded discs . . . . *var. diffluens*.

#### SUMMARY.

In the first part of the paper an account of the structure and development of *Tetrasporidium javanicum* is given. The thallus consists of two

perforated layers of cells, provided with numerous connecting processes. The sporangia described by Moebius are shown to be due to the attack of a species of *Vampyrella*. In view of the absence of pseudocilia the genus cannot be referred to Tetrasporaceae, but must find a place among Palmellaceae.

The second part of the paper deals with the genus *Ecballocystis*, a number of species of which are described from various parts of India, while two South African species are also taken into consideration. The diverse form of the colony in the different species is related to the varying behaviour of the daughter-cells after division, which is invariably oblique. The cells contain from two to many discoid parietal chloroplasts with pyrenoids, and usually exhibit a marked polarity. No evidence of motile stages or resting spores has been obtained. The normal method of reproduction would appear to be by means of detached daughter-cells, already enveloped by a membrane at the time of liberation. In some species such cells are produced in considerable numbers within the mother-cell. When floated on to a suitable substratum, the cell secretes mucilage at one pole and gradually becomes erected at right angles to the substratum. It is pointed out that *Ecballocystis* is not closely related to the other genera of Chlorodendrales with which it has usually been associated, and a possible affinity with *Oocystis* is indicated.

The author wishes, in conclusion, to express his great indebtedness to Professor F. E. Fritsch for the plentiful advice he has given during this investigation, and for assistance in preparing the paper for press. He also wishes to acknowledge the help afforded by Dr. N. Carter and Miss F. Rich.

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## DESCRIPTION OF PLATES VII AND VIII.

Illustrating Professor Iyengar's paper on Two Little-known Genera of Green Algae  
(*Tetrasporidium* and *Ecballocystis*)

### PLATE VII.

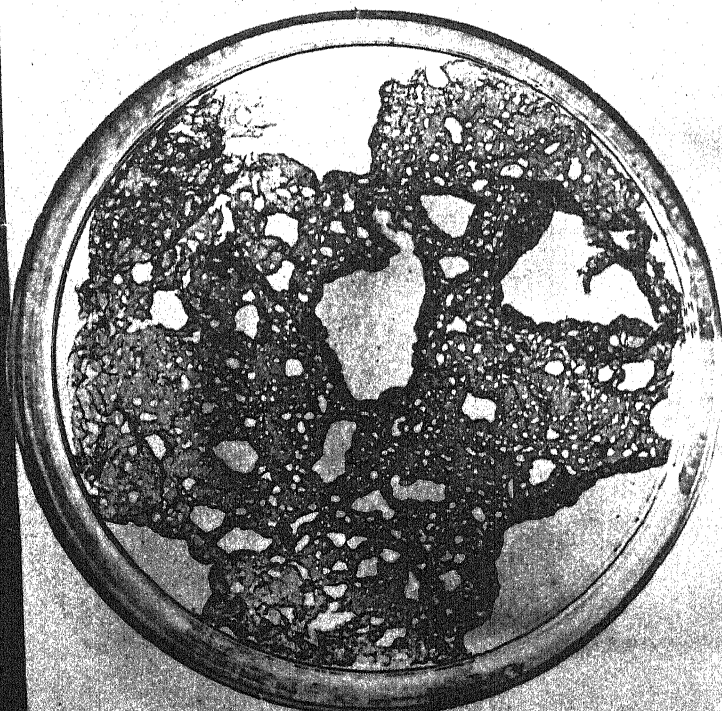
*Tetrasporidium javanicum*, Moeb.

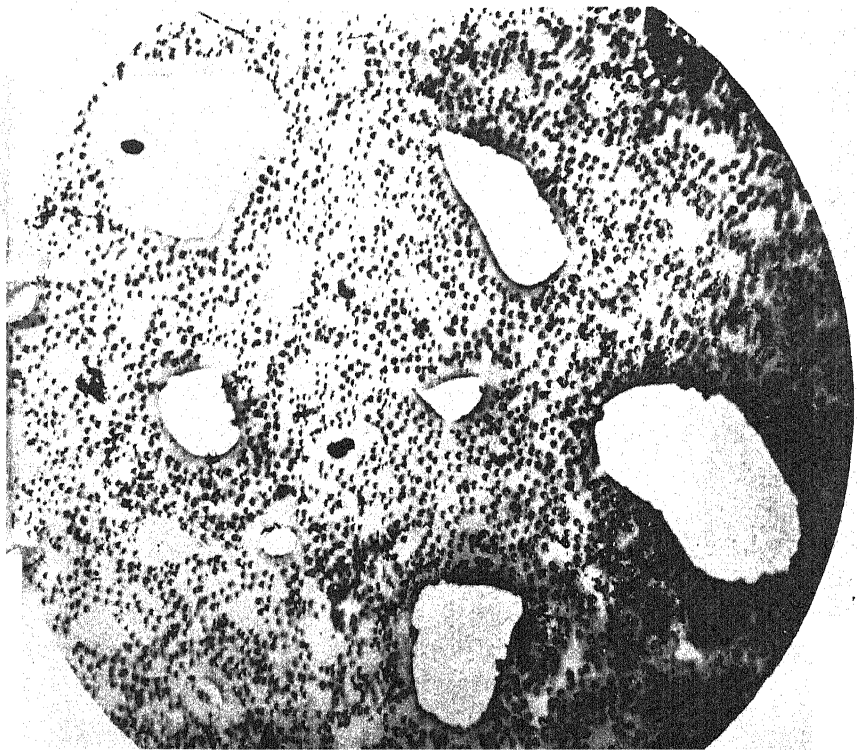
- Fig. 1. Photograph of a thallus floated in water in a Petri dish (about half nat. size).  
 Figs. 2, 3, 4. Photomicrographs of portions of the thallus. Fig. 2 shows the two perforated layers, the lower seen faintly below the upper, unstained.  $\times 75$ .  
 Fig. 3. Part of a single layer of the thallus, lightly stained with safranin.  $\times 150$ .  
 Fig. 4. Portion of the thallus showing the two layers with cross-connexions, lightly stained with safranin.  $\times 150$ .

### PLATE VIII.

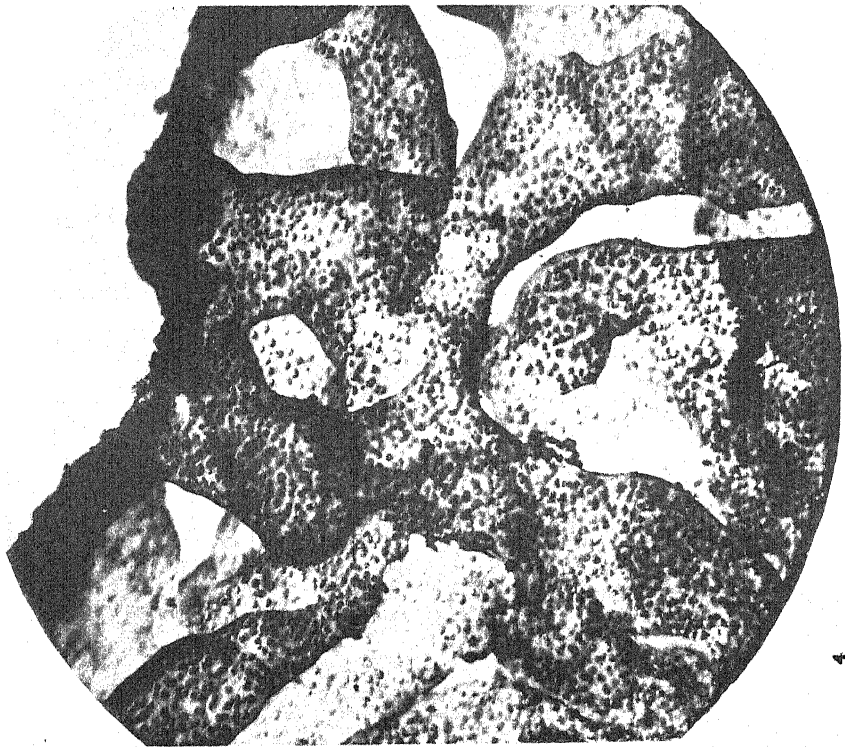
*Ecballocystis*, Böhlin.

- Figs. 5 and 6. Complete colonies of *Ecballocystis courtallensis* f. *jögensis*. Fig. 5  $\times 60$ ; Fig. 6  $\times 110$ .  
 Fig. 7. A portion of the colony shown in Fig. 2, more highly magnified.  $\times 380$ .  
 Fig. 8. Photomicrograph of a microtome section of *E. fritschii* showing the cells crowded near the periphery of the thallus.  $\times 65$ .  
 Fig. 9. Young colonies of *E. courtallensis* f. *typica*.  $\times 100$ .  
 Fig. 10. Three cells of *E. courtallensis* occupying the end of a branch; the upper two cells have been produced by division of a sister-cell of the lowest which has not divided; the chloroplasts are clearly seen in the upper two cells.  $\times 600$ .



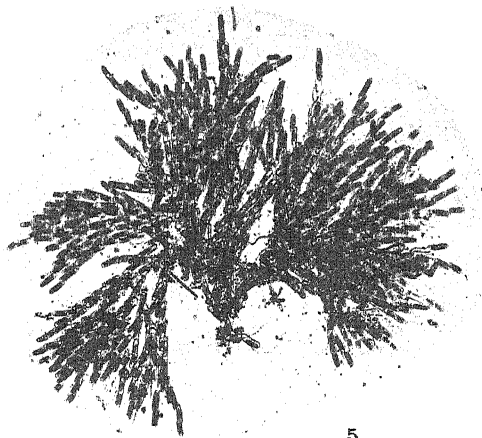


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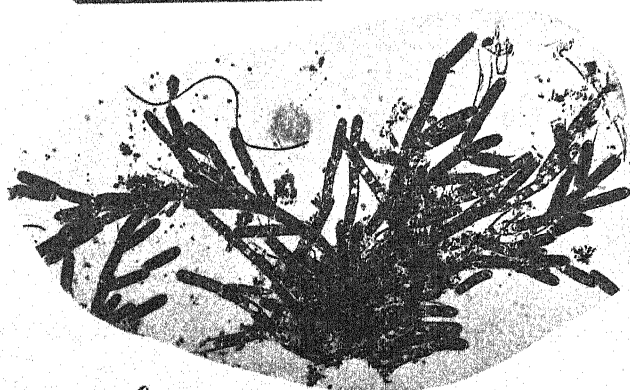
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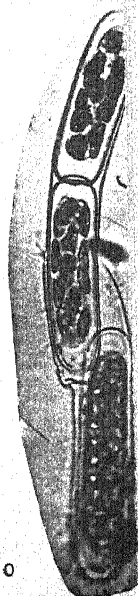
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# Observations on the Pycnidium of *Botryodiplodia theobromae*, Pat.

BY

C. W. WARDLAW.

(Imperial College of Tropical Agriculture, Trinidad, B.W.I.)

With Plate IX and eleven Figures in the Text.

## I. INTRODUCTION.

THE position regarding the classification of fungal organisms of the *Diplodia* group has been admirably summarized by Nowell (4) as follows: 'Nomenclature in the genus *Diplodia* is particularly confused as the result of attempts to divide up its species amongst smaller genera: *Lasiodiplodia*, *Botryodiplodia*, *Chaetodiplodia*, &c., based on characters that have proved to be unstable.' The well-known species *Botryodiplodia theobromae*, Pat., which has a wide distribution in the tropics as a saprophyte and partial parasite on many hosts, recently came to the writer's notice as an important parasite on bananas during storage (9, 10). In the course of an investigation of the physiology of the fungus it was observed that, in relation to the cultural conditions provided, the fructifications displayed a very considerable range of variation, from small separate flask-shaped pycnidia without a stroma, to large and conspicuous stromata in which pycnidia, of varying shape, were embedded. Thus, for example, the fructifications which develop on fallen fruit in the field, or on cold storage fruit, are essentially small, separate, individual pycnidia. If conidia or tissue inocula from such material are placed on steamed potato blocks or on synthetic media rich in carbohydrates, large stromatic bodies, by which the organism is readily recognized, soon appear in considerable numbers. Similarly in specimens of cocoa-pod rot Nowell (4) describes the pycnidia as black, carbonaceous, more or less rounded, and immersed in the cortex; he also says that they may be combined in groups or sometimes in a stroma, and that they become pilose when grown in moist air. On the sugar-cane Howard (3) describes the pycnidia as separate flask-shaped structures which develop under the rind. The question of synonymy has been extensively dealt with by Petch (5) and need not be reconsidered here. The

following list (5) of synonyms, by which the fungus has been described, is indicative of the variation in pycnidial development which has been observed :

- Botryodiplodia theobromae*, Pat., 1892.
- Macrophoma vestita*, Prill et Del., 1894.
- Diplodia cacaicola*, P. Henn, 1895.
- Lasiodiplodia nigra*, App. et Laub., 1906.
- Botryodiplodia elasticae*, Petch, 1906.
- Chaetodiplodia grisea*, Petch, 1906.
- Lasiodiplodia theobromae*, Griff. et Maub., 1909.
- Diplodia rapax*, Massee, 1910.

From the writer's observations there is also good reason to believe that the species recorded as *D. Musae*, Died., and described by Sydow and Butler (8) from dead fruits of *Musa sapientum* in Assam is identical with *B. theobromae*, Pat.

One of the important systematic characters used to diagnose organisms of the *Diplodia* type depends on whether the pycnidia are separate or united in a stroma (2, 6, 7). In *B. theobromae*, at least, the experimental evidence shows that this character is subject to variation according to growth conditions.

## II. TYPES OF PYCNIDIAL FORMATION.

A brief account may now be given of the several types of fructification observed.

When a *Botryodiplodia* infection develops on bananas in cold storage at 53° F., the pycnidia are small, separate, round, black, and carbonaceous, flask-shaped structures with a short straight neck. From the earliest stages these appear on the epidermis as superficial structures, or at most very slightly sunken (Text-fig. 1, and Pl. IX, Figs. 1 and 2). They are produced in very large numbers, and form a black crust over the diseased fruit. Under more humid conditions superficial, vegetative mycelium develops over the surface of affected fruits, and the pycnidia already formed become pilose. When the superficial mycelium develops abundantly, pycnidia which are still immature become abortive, being completely submerged by the mat of vegetative hyphae (Text-fig. 6). Similar pycnidia but rather more sunken (Text-fig. 7) were also observed when the disease occurred on fallen fruit in the field.

The pycnidium consists of a thick wall of hyphae, dark and carbonaceous on the outer side and lighter coloured within. From a darkly-staining layer of hyphae, lining the inside of the flask, conidia, borne on conidiophores, and paraphyses arise. The conidia seem to be extruded mostly in

the non-septate condition, mature, uni-septate, brown-coloured conidia being found in abundance outside the ostioles.

When grown on synthetic or other sterilized media which provide a sufficient concentration of carbohydrate, large and conspicuous stromata are usually formed. The extent of this development, however, depends on the composition of the culture medium and other factors, and considerable variation in the form of the stromata may be observed.

As an account of the physiology of this fungus is in the course of preparation, the various culture media employed need not be described in detail here. The general observation made was that *B. theobromae* possesses a marked capacity for growth on high concentrations of starch, sucrose, and other carbohydrates, on which media stromata are produced in abundance. At the lower concentrations of starch or sucrose (e.g. 1 per cent. starch as in Brown's (1) Standard Medium) pycnidia and stromata were very scantily developed or absent. The following media were prepared:

	Medium.	%.
Asparagin . . . . .	2.00 gm.	1. 0.2 starch
K <sub>3</sub> PO <sub>4</sub> . . . . .	1.25 "	2. 1.0 "
MgSO <sub>4</sub> . . . . .	0.75 "	3. 2.0 "
Agar . . . . .	15.00 "	4. 4.0 "
Water . . . . .	1000 c.c.	5. 0.2 sucrose
		6. 1.0 "
		7. 2.0 "
		8. 4.0 "

After forty-two days on medium No. 1 no pycnidia were present; on No. 2 a few small cake-like stromata had appeared, while on Nos. 3 and 4 many large stromata had been produced. A similar series was obtained from the sucrose media. On the latter, pycnidia and stromata were formed at concentrations as high as 75 per cent.

From further experiments it was found that the nature and extent of stroma formation varied according to the carbohydrate concentration, sucrose media being superior to starch media. Some results obtained with the standard medium, as above, and different sucrose concentrations, are summarized below.

At the more favourable concentrations, pycnidial primordia could be observed round the central inoculum on the third day, and in the course of 10 to 15 days large stromata, 1 cm. in height and 0.2-0.5 cm. in diameter, had developed. The extrusion of conidia was in evidence after 15 to 20 days.

When a medium of suitable composition was poured as a shallow layer in a deep Petri-dish, the stromata developed as cylindrical structures, one or more cm. long, with a sinuous column indicative of a number of growth accretions (Pl. IX, Fig. 3). These were pilose at first, but they frequently became smooth on account of condensation water. Sometimes they

remained as simple cylinders bearing sunken pycnidia at the distal end, but frequently they forked once or twice, and individual pycnidia or small groups of pycnidia were found in each arm (Pl. IX, Fig. 4). The largest fructifications observed were those in which the developing pseudo-tissue had widened out so as to form a large conical stroma (Pl. IX, Fig. 5) with a broad distal surface bearing a few or many pycnidia (Pl. IX, Fig. 6). The fertile distal end sometimes showed a pilose involuted margin within which lay the sunken pycnidia. In mature specimens the presence of the latter was indicated by the extrusion of masses of conidia (Pl. IX, Figs. 4 and 5). )

Concentration  
of sucrose  
per cent.

Development of mycelium and stromata.

0.5	Mycelium feebly developed and of pale grey colour. Stromata very small and few in number or absent.
1.0	Mycelium more abundant and of greyish green colour. Very feeble pycnidial development round centre and periphery.
2.0	Mycelium abundant and of dark green colour. Pycnidial primordia plentifully developed round centre after three days. Older cultures had a central cluster of large stromata standing out clearly from the aerial mycelium.
5.0	Resembles the 2.0 per cent. medium, but pycnidia, and later, stromata, developed over a larger part of the surface.
10-12	Approximately the optimum for stromatic formation. Numerous primordia present at end of third day. Stromata formed over entire surface and standing out clearly from the aerial mycelium.
12-75	Stromata progressively more slowly formed and tend to be overgrown by the dense mat of aerial mycelium.

( In microtome sections it was found that the stromatic pycnidia were very variable as regards number, size, and shape. Sometimes only a few pycnidia were present (Pl. IX, Fig. 6), while in others the distal end was a mass of fused pycnidia (Text-fig. 8). The structure of the stroma showed a number of points of interest. For example, in stromata which had been carefully removed, pycnidia showing arrested development were found at the extreme base, at the point of contact with the culture medium (Pl. IX, Fig. 6). These appeared to be semi-abortive, and contained a few mature but frequently abnormal, uni-septate brown conidia, which had been unable to escape. The pycnidium thus constitutes the primordium on which the large cylindrical or conical stroma is built up. Such specimens are of especial interest in that they indicate the way in which the transition from the simple to the more complex type of fructification has taken place. In Text-fig. 9 a longitudinal section is shown of a stroma in which pycnidia have developed at four different levels, namely, at the base of the stroma (*a*), and successively higher up at (*b*), (*c*), and (*d*). The pycnidia at (*a*) and (*b*) were semi-abortive, being completely occluded by the surrounding pseudo-

parenchyma. They contained a few uni-septate brown conidia. Those at (c) were still immature, while the last-formed pycnidia at (d), situated at the distal end, showed the most advanced development and normal structure.

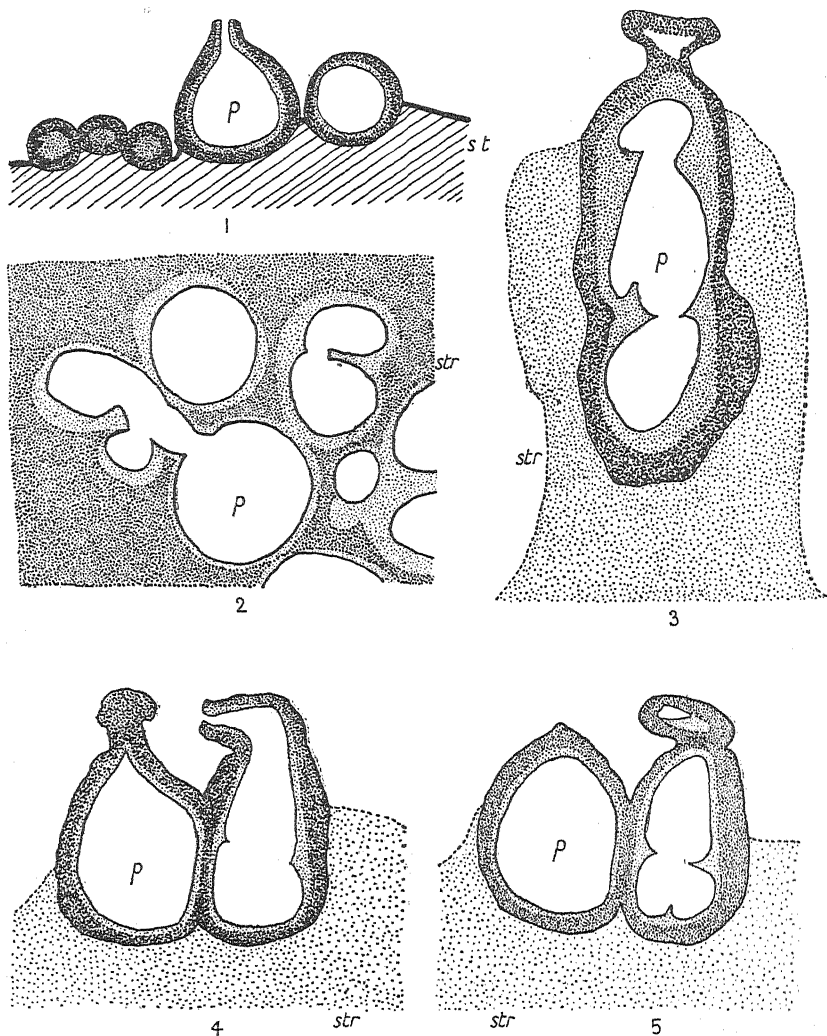
These observations were followed up by an examination of young cultures on different media before stromata had commenced to form, and it was usually found that small, separate, immature pycnidia were distributed over the surface of the medium, but frequently hidden by aerial hyphae. Thus, on synthetic culture media, separate pycnidia of the usual *Diplodia* type are the first to be developed, and they constitute the primordia on which the large stromatic fructifications of the *Botryodiplodia* type will be formed. On the standard medium in which carbon was supplied in the form of mannitol (2.5 per cent.), it was found, from microtome sections of young and old cultures, that pycnidia of the *Diplodia* type had developed, but that compound pycnidia and stromata had not. Again, on Richards's solution agar, young cultures showed numerous separate pycnidia. In older cultures large stromata did not appear, but small compound fructifications, consisting of a rosette of secondary pycnidia growing round the original one, were sometimes observed.

The pseudo-tissue of the stroma consists of uniform interwoven hyphae of moderate compactness, here and there interrupted by the presence, at definite levels, of large lacunae filled with less compact hyphae. The arrangement and shape of such lacunae (Pl. IX, Fig. 6) suggests that they may represent pycnidial cavities which have been modified at an early stage by the rapid upward growth of the stroma, but a definite opinion on this point cannot yet be put forward.

The pycnidia which occur at the distal end of these large stromata are usually sunken or occluded to some extent. They may occur singly, when the normal structure is clearly seen (Text-fig. 11), or they may be so closely aggregated together that the typical flask-shaped structure is entirely lost (Text-figs. 2 and 8). The normal pycnidium found in such stromata has the characteristic round or ovoid shape, with a short straight neck, but is usually of much larger size than that obtained from the surface of diseased fruits.

In stromata which had developed to a large size, normal pycnidia like those described above were not always observed. Those on sucrose-rich media were found to possess a high degree of plasticity, and marked variation was observed in the shape of the conidial cavities (Text-figs. 2, 3, 5, and 8). In Text-fig. 3 it will be seen that the pycnidium is a long cylindrical structure with an irregular cavity whose appearance suggests a fusion of several pycnidia. This is attributed to the continued growth of the stroma at a time when the formation of pycnidia was in progress, the immature pycnidia participating in the general growth by elongation and the

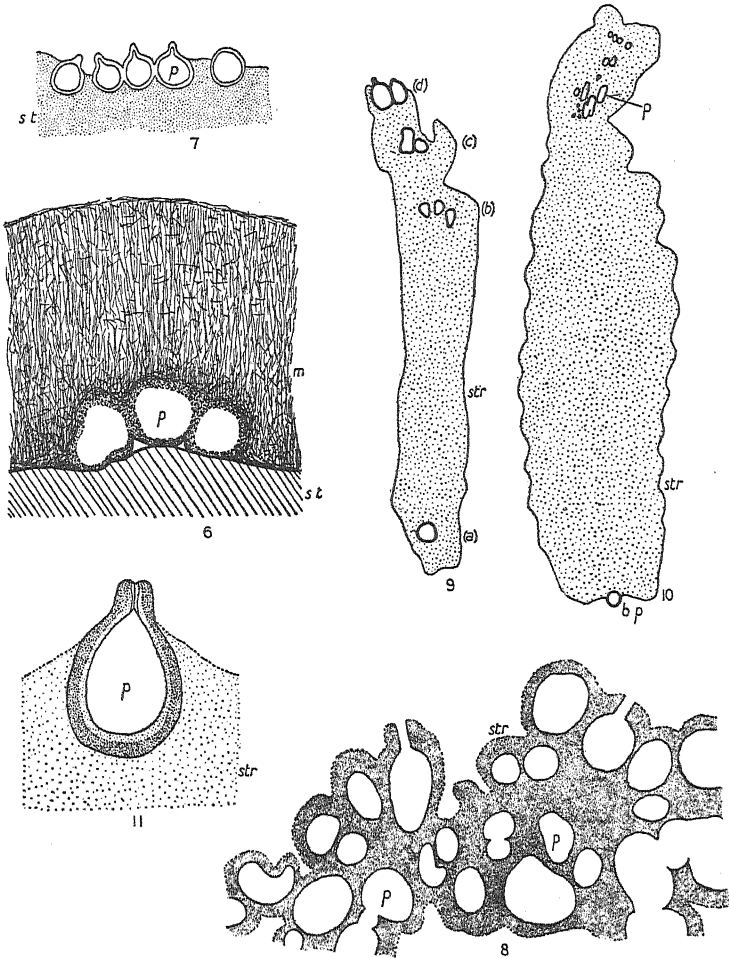
formation of additional conidial cavities. Thus elaborate and irregular stromatic pycnidia are formed (Text-fig. 2), consisting essentially of a number of spherical cavities joined together under a system of continued growth.



TEXT-FIGS. 1-5. 1. Pycnidia of *Botryodiplodia theobromae*, Pat., on the surface of banana skin (cold storage fruit); *s.t.*, skin tissue; *p.*, pycnidium ( $\times 55$ ). 2-5. Types of pycnidia (*p.*) developed on stromatic fructifications; *str.*, pseudo-tissue of stroma.  $\times 55$ .

In the small pycnidia developed on fruit the neck as a rule is straight. On the stromatic pycnidia, on the other hand, more often than not the neck was found to be bent or twisted as in Text-fig. 4, presumably in relation to some external stimulus. Text-fig. 5, a companion serial section to Text-fig. 4, shows a characteristic involution of the spore cavity.

On a concentrated sucrose medium (20 per cent. sucrose) fructifications of the same general type as those described above were observed. Many of the stromata, however, remained short and flattened (Pl. IX, Fig. 7). The



TEXT-FIGS. 6-11. 6. Immature pycnidia (*p.*) on surface of banana skin (*s.t.*) overgrown and rendered abortive by a thick web of mycelium (*m.*).  $\times 55$ . 7. Partly submerged pycnidia (*p.*) found on skin (*s.t.*) of fallen fruit in the field.  $\times 33$ . 8. Transverse section through a large stroma showing the abundant confluent pycnidia; *p.*, pycnidium; *str.*, pseudo-tissue of stroma.  $\times 33$ . 9-10. Longitudinal sections through large stromata, showing pycnidia developed at different levels (*a*), (*b*), (*c*), (*d*); *b.p.*, basal pycnidium; *str.*, stroma.  $\times 8$ . 11. Normal pycnidium (*p.*) developed on a stroma (*str.*).  $\times 55$ .

upper surface, on which numerous sunken pycnidia could be observed, had, as a rule, a well-marked involuted margin (Pl. IX, Fig. 8). Longitudinal sections through this type of stroma showed that, in addition to the pycnidia on the upper surface, others were distributed in one or two layers

within. On media of low starch or sucrose concentration on which only a few fructifications were formed, the stromata obtained were small and cake-like, with conspicuous pycnidia (Pl. IX, Figs. 9 and 10).

### III. THE INFLUENCE OF LIGHT.

In Petri-dish cultures absence of light resulted in a more or less complete suppression of pycnidia, and the mycelium also failed to develop its usual dark greenish colour. From time to time cultures on carbohydrate media were placed in darkness and examined after 5, 10, and 15 days. There was an entire absence of stromata in all cases, although on similar media exposed to daylight in the laboratory they were plentifully developed. After 12 days' growth in total darkness some cultures were exposed to daylight for 10 days, but no pycnidia or stromata developed at any point on the surface of the medium. In cultures with columnar fructifications it was noticeable that these were positively heliotropic so that they inclined towards the source of illumination.

While laboratory experiments indicate the importance of light in the formation of pycnidia and stromata there is some evidence which suggests that this may not always be so. Thus on diseased fruit from storage rooms which are kept in darkness, except for short spells of feeble illumination, numerous small pycnidia are formed, and the latter have also been observed in abundance on fallen fruit sheltered from light by vegetable debris.

### IV. DISCUSSION.

In the observations and illustrations submitted here, it has been shown that the fructifications of *B. theobromae* are of a plastic nature and subject to considerable variation under different growth conditions. Thus the formation of the large stromata is directly correlated with the growth made by the fungus under suitable conditions of humidity and nutrition. It has been found that pycnidial primordia on the surface of bananas may become arrested by a heavy overgrowth of aerial mycelium developed in relation to increased atmospheric humidity. It has also been seen that the large stromatic fructifications consist essentially of a basal pycnidium, or collection of pycnidia, on which has been superimposed a mass of rapidly growing pseudo-tissue, the basal primordia being rendered semi-abortive as a result. The rapid formation of pseudo-tissue is referable to the capacity for exploiting concentrated carbohydrate media which *B. theobromae* possesses in a high degree. It has further been noted that pycnidia tend to be formed successively at different levels on the upwardly growing stroma, and that the individual pycnidium is itself of a plastic nature so that the characteristic rounded conidial cavity is frequently replaced by an elongated and involutioned one.



The facts for *B. theobromae* indicate that the organism is, at least during the earlier stages, essentially of the simple *Diplodia* type, and that the more complicated stromatic fructifications (on which the genera *Botryodiplodia* and *Lasiodiplodia* have been raised) are definitely referable to growth conditions. It does not follow that this generalization will hold good for all members of this group since, in some cases, it is possible that the more elaborate type of fructification, once evolved, may become more or less definitely fixed. The data submitted here suggest that useful light might be thrown on the morphology and classification of the whole group by suitable experimentation with other forms.

#### V. SUMMARY.

1. Nomenclature in the *Diplodia* group of fungi is confused as a result of attempts to classify its members into different genera based on characters which are unstable.
2. An account is given of the various types of fructification produced by *B. theobromae*, Pat., when grown under different conditions. The pycnidia may be separate and simple, or they may be united into more or less elaborate stromatic fructifications according to humidity and nutritional factors.
3. Light is important in the development of the stromata which are positively heliotropic.
4. In a brief discussion the fundamental nature of the stromatic fructifications is considered.

The author has much pleasure in expressing his thanks to Professor H. R. Britton Jones and Professor E. E. Cheesman for kindly providing technical facilities.

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## EXPLANATION OF PLATE IX.

Illustrating Dr. Wardlaw's paper on Observations on the Pycnidium of *Botryodiplodia theobromae*, Pat.

Figs. 1-6. Different types of fructifications of *Botryodiplodia theobromae*, Pat. All drawn to same scale.  $\times 5$ .

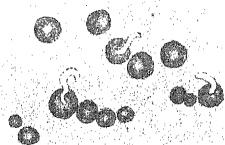
Fig. 1. Separate pycnidia on banana skin. Some show extrusion of conidia.

Fig. 2. Section through same material.

Fig. 3. Stromatic fructifications developed on different concentrations of carbohydrate; 4 and 5 show extrusion of conidia.

Fig. 6. Longitudinal section showing basal and distal pycnidia and lacunae.

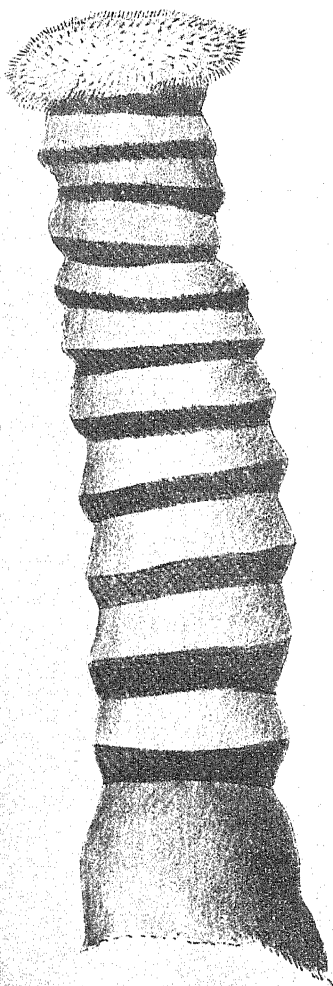




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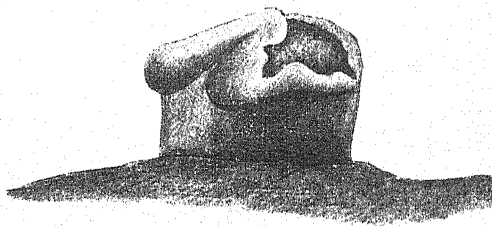
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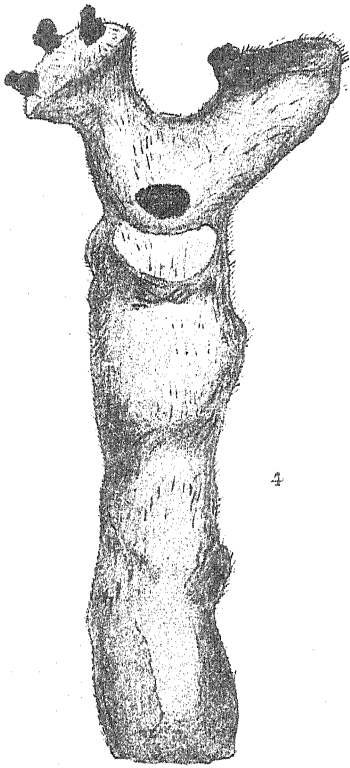
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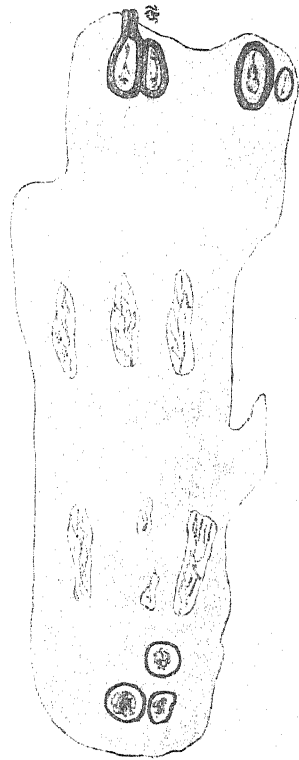
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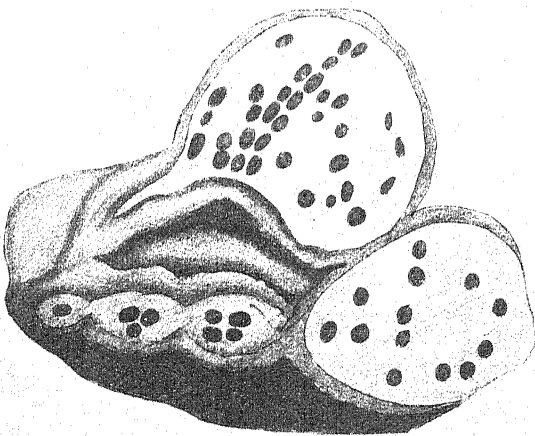
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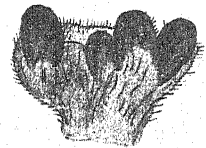
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# On Carpel Polymorphism. V.

BY

EDITH R. SAUNDERS.

(Sometime Fellow of Newnham College, Cambridge.)

With eighty-eight Figures and nine Diagrams in the Text.

THIS account deals with the problems presented by the gynoecium in the Caryophyllaceae, Primulaceae, and Myrsinaceae.

## CARYOPHYLLACEAE (Figs. 1-56 and Diagrams A-D).

The characteristic feature of the gynoecium of the Caryophyllaceae, viz. the free central mode of placentation, was examined early in the course of the present study of the carpel in the case of *Dianthus* and *Lychnis* (7), pp. 142-3, in which the carpel number had previously been accepted as 2 and 5 respectively. It was shown that the number in these two genera is, in fact, 4 and 10. It followed from these observations that throughout the family the gynoecium must be regarded as constructed of two carpel whorls, the outer members being of the valve type and sterile, the inner, consolidated and fertile, and hence that the number of carpels in all cases is twice that hitherto stated. For those forms such as *Lychnis* and *Cerastium* which are pentamerous throughout we shall therefore write  $G_5 + 5$ . But among such isomerous forms we meet with an unexpected diversity in the ground-plan of the gynoecium, for in some types the loculi, separate below, though becoming continuous above, stand opposite the sepals, and in others in front of the petals. Concerning these diametrically opposed relations Eichler, unable to arrive at any solution, found himself obliged to admit that the facts have to be accepted but that the explanation must be left to the future (3), p. 111. No advance on this statement of the position has so far been attempted. On the view that the gynoecium consists of a single carpel whorl none, indeed, seems sustainable. On the other hand, a rational explanation of this anomaly at once presents itself when it is appreciated that the gynoecium is a 2-whorled structure. In considering the evidence for this interpretation it will be convenient to deal first with some illustrative cases from the section Silenoideae, in which the flowers are usually large with both stamen whorls complete.

## I. SILENOIDEAE. Calyx gamosepalous. (Figs. 1-46 and Diagrams A-D).

Examination of a number of types belonging to the subsection *Lychnideae* brought to light the fact that in those forms which are isomerous throughout and have the loculi in line with the sepals (many species of *Lychnis*) either a gynophore<sup>1</sup> is developed (*L. coeli-rosa*, Desr., *L. chalcidonica*, L., *L. Haageana*, Lem.) or some other modification having a like effect is present (*L. Flos-Jovis*, Desr.); whereas in isomerous types in which there is no gynophore or other compensating modification (*Lychnis* (*Agrostemma*) *Githago*, Scop., and *Cerastium*) the loculi stand in line with the petals. It is this association of seemingly entirely disconnected morphological characters which points the way to an understanding of the varying position of the loculi. In order to make this connexion clear it is necessary to follow the course of events during development of *all* the floral whorls in the two cases. As a typical example of the first category (loculi antesealous) we may take *Lychnis coeli-rosa*, Desr. (Figs. 1-14), which possesses a well-developed gynophore. At the top of the flower stalk are to be seen passing out from the central cylinder five vascular cords which become the midribs of the sepals, and almost simultaneously, on the intervening radii, five trunk cords which supply the bundles for the commissural ribs of the gamosepalous calyx, which in *Lychnis* is 10-ribbed. These latter bundles carry out conjoined with them for a short distance the bundles for the five petals. Only after the emergence of these ten cords does elongation of the gynophore take place. As the trunk cords break up the petal bundles turn upwards and continue in this direction in the ordinary way, but the ten bundles for the calyx pursue a descending course for some distance down the internode below (= region of gynophore), and only then turn outwards and upwards into the calyx tube, which is exerted at the level of the bend. Transverse sections taken between top and bottom of the gynophore show the ten sepal bundles (five median and five commissural) cut twice—in their downward course in the gynophore and again in their return upward course in the exerted calyx tube (Figs. 2, 3, and 14).

Now this reversal of the normal direction of the sepal bundles and accompanying downward displacement of the exertion level of the sepals will have the result of removing, or at least relieving, the restraining effect

<sup>1</sup> The gynophore may arise either by the development of a proper internode extending up from the level of origin of the bundles for the sepals (which are here soon exerted) to the level of exertion of the corolla; or through the downward displacement of the exertion level of the calyx and the consequent separation of this level from that of the corolla exertion by a corresponding portion (but a portion only) of the internode below (see Fig. 14).

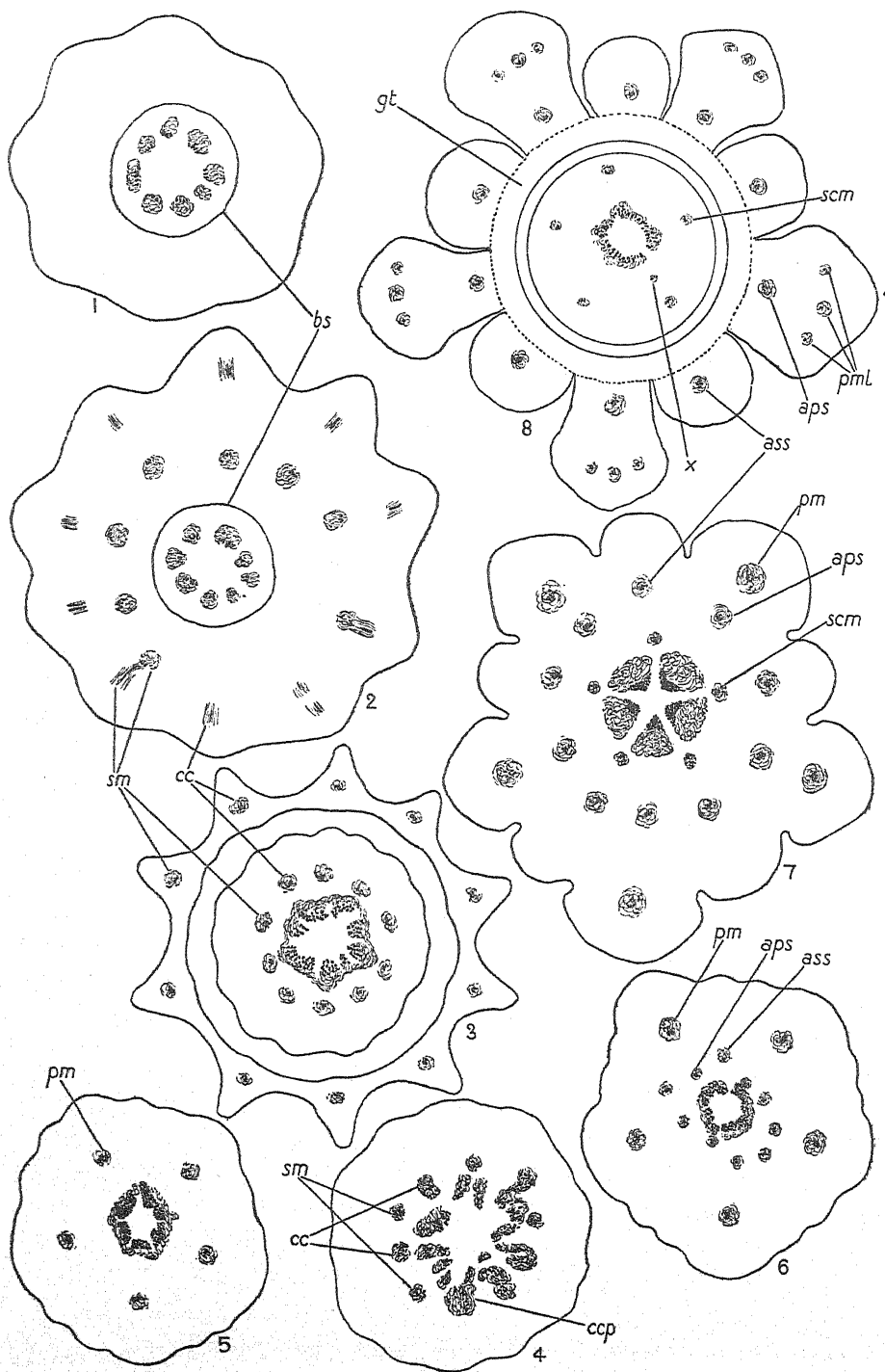


which we may suppose is ordinarily exerted when rapid development of the successive whorls occurs in the ordinary manner. For when a gynophore is present there will be no engirdling mass of calyx tissue to hinder the bundles of the superposed whorls from turning outwards to the periphery in their proper sequence. Still further relief in this direction is possibly afforded by the development of the stipe, which allows radial extension of the carpels to take place at a level at which the flower tube, already disjoined from the gynoecium, has considerably enlarged.

I have elsewhere suggested that this limiting factor of space may well have been a main cause in also bringing about consolidation of the carpels in the first instance (13), consolidation frequently taking place in such a manner as to bring obdiplostemony in its train, with accompanying development of the loculi in line with the petals. It will be recalled that the Caryophyllaceae as a family are exceptional in that isomerous forms with a full (6-whorled) ground-plan are, nevertheless, not as a rule obdiplostemenous. *They do not exhibit this condition because the two carpel whorls extend equally far outwards from the centre.* This equal radial extension of both whorls is as general throughout this family as it is exceptional in isomerous types among the Geraniales (10, 12, p. 116). In this latter Order obdiplostemony associated with a more central disposition of the less extended antesepalous carpel whorl, and consequent antepetalous position of the loculi, is the rule. Only in *Linum* and its allies, in which both whorls, alike in form, extend equally far from the centre and come to stand in a single ring, are the stamens not obdiplostemenous.

As mentioned above, in those isomerous Silenoideae which have the full number of floral whorls and which develop a gynophore with calyx bundles following at first a downward or horizontal course, the loculi are found to be antesepalous. Assuming space conditions to take effect in the manner described above, this is the arrangement we should expect. For with the removal of the restraint presumably imposed upon the inner whorls when they develop within the limits set by an encompassing calyx, the midrib bundles for the staminal and carpel whorls turn outwards in their proper sequence. Hence resistance to radial expansion of a whorl of valve carpels will naturally be least on the radii proper to the next (alternating) whorl, i.e. the radii of the sepals, consequently the loculi arise in a line with these members.<sup>1</sup> It may be mentioned in this connexion that in some species of *Lychnis* otherwise similar to *L. coeli-rosa* in the characters described above, the fertile carpels no longer develop midrib bundles, the whole of the residual vascular tissue on the corresponding radii continuing upwards in a central position and becoming differentiated

<sup>1</sup> Although these interrelations between the several whorls lend themselves most readily to statements in terms of *space*, they might no doubt with equal propriety be expressed in terms of *time* (*rhythm*).



FIGS. 1-8. Caryophyllaceae. *Lychnis coeli-rosa*, Desr. All from transverse sections taken at successively higher levels. 1. Flower stalk. In the centre the vascular cylinder surrounded by a well-marked bundle sheath. 2. Flower base near the level of exsertion of the calyx (see *a* in Fig. 14). All but the one bundle (in the middle line below) of the ten median and commissural bundles for the sepals are seen cut through twice, transversely, as they descend the length of the gynophore (inner circle) and again obliquely, as they turn outwards preparatory to passing up into the exserted gamosepalous calyx. The bundle cut once only has been caught as it makes the bend at the change of direction. In the centre the residual vascular ring and bundle sheath. 3. The same above the level of exsertion of the calyx and below that at which its bundles issue from the central cylinder (see *b* in Fig. 14). The exserted calyx tube with alternate midrib and commissural bundles encircles the gynophore, which shows the same ten bundles in their earlier downward course forming a ring round the central five-sided vascular figure. [For convenience the calyx tube has been reduced in size in proportion to the gynophore.] 4. The same at the level at which the five sepal midrib bundles and the five trunk cords which shortly differentiate into the commissural bundles and the petal midribs leave the central cylinder (see *c* in Fig. 14). 5. The gynophore above the level of origin of the calyx bundles showing the five detached petal bundles and the residual central cylinder (see *d* in Fig. 14). 6. The same at the level of origin of the ten bundles for the stamens. 7. The same showing the bundles for the five petals, ten stamens, and the five antepetalous sterile carpels arranged in concentric rings. In the centre the residual vascular masses serving the five antepetalous fertile carpels. 8. Ovary stipe surrounded by the corolla-stamen tube from which it is now disjoined. In the stipe the midribs of the five antepetalous sterile carpels have turned out from the central ring. An additional small strand ( $\times$ ), which has been carried out for a short distance and is later reabsorbed in the residual complex, is seen below on the right.

To avoid repetition the complete list of the abbreviations used in explanation of the whole set of figures is given here:

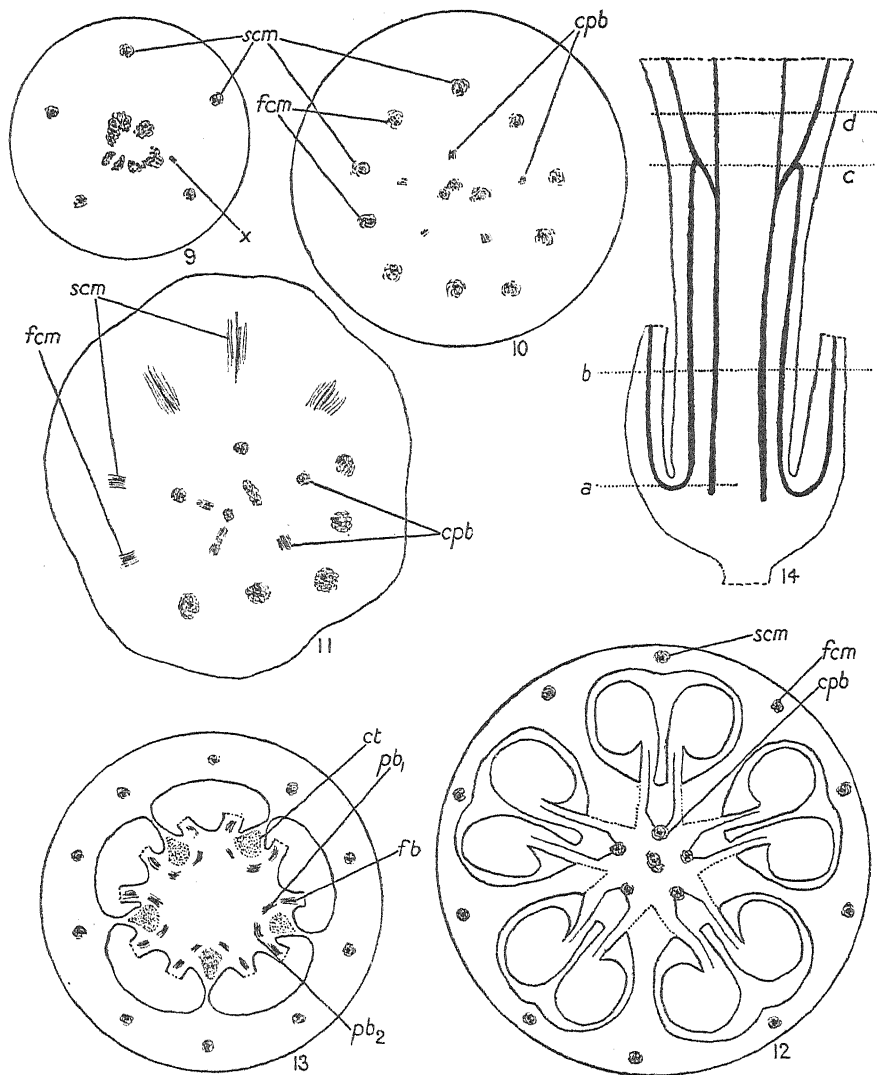
- a.p.s.*, antepetalous stamen bundle.
- a.s.s.*, antesepalous stamen bundle.
- b.s.*, bundle sheath.
- c.c.*, calyx commissural bundle.
- c.c.p.*, calyx commissural + petal midrib trunk cord.
- c.c.p.s.*, calyx commissural + petal-stamen trunk cord.
- c.c.p.s.c.*, calyx commissural + petal-stamen + carpel trunk cord.
- c.p.b.*, compound placental bundle.
- c.t.*, conducting tissue.
- f.b.*, funicle bundle.
- f.c.m.*, fertile carpel midrib.
- g.t.*, glandular tissue.
- l.*, loculus.
- ov.*, ovale.
- p.b.<sub>1</sub>*, *p.b.<sub>2</sub>*, twin placental bundles of a single fertile carpel.
- p.m.*, petal midrib.
- p.m.l.*, petal midrib and laterals.
- p.s.*, petal-stamen trunk cord.
- p.s.c.*, petal-stamen-carpel trunk cord.
- s.c.m.*, sterile carpel midrib.
- s.m.*, sepal midrib.
- s.s.*, sepal-stamen (or -staminode) trunk cord.
- st.*, stamen bundle.
- x.*, carpel bundle of doubtful significance.

into the several placental bundle masses. In such species as, e.g. *L. chalconica*, L., the radial dimension of these carpel members is not diminished thereby, hence the position of the stamens is not affected (Fig. 15).

As illustrative of the opposite category, viz., the isomerous type with the loculi in line with the petals, we may take *Lychnis (Agrostemma) Githago*, Scop. (Figs. 16–18). Here there is no appreciable interval between the exertion level of calyx and corolla and scarcely any stipe to the ovary. So far as the four outer whorls are concerned, the vascular cords arise in their proper order on alternate radii, as in the preceding class, those for the petals being carried out, as before, conjoined with the commissural bundles of the sepals. But in this species these latter bundles and the midrib bundles of the sepals turn upwards from the outset. That is to say, we have in this type the conditions which, as suggested above, offer increasing resistance to the radial extension of successive whorls. After the outturning of the bundles for the perianth has been followed by that of the bundles for the two sets of stamens, this resistance appears to be sufficient to bring about a momentary check to radial development. The growth impetus, we may suppose, travels on laterally and upwards. Time and (vertical) space thus gained, radial extension again becomes feasible, but now takes place on the other set of radii, for here it is the antepetalous carpels which expand to accommodate the loculi. The antesepalous members thus come to constitute the inner whorl.

Not only does this conception of the effect of space restriction on the order of development of the innermost floral whorls provide an answer to the hitherto unsolved problem of the varying position of the loculi in the types cited, but it furnishes us with the required clue in other cases not coming under the two categories discussed above. For example, since a gynophore is lacking in *Lychnis dioica*, L., it might be presumed that, as in similar circumstances in *L. (Agrostemma) Githago*, the loculi would stand in line with the petals, whereas, in fact, they lie in front of the sepals. But although in *L. dioica* ♀ (Figs. 19–27) ten bundles for the androecium are actually present (Fig. 21), they are but slightly developed and no corresponding staminal members are formed. The situation relieved by the non-development of both stamen whorls, the carpels arise in their due position and sequence notwithstanding the absence of a gynophore.

Similarly we are able to account for the carpel ground-plan of *L. Flos-Jovis*, Desr., which appears at first sight not to accord with expectation based on the relations observed to hold in the other species dealt with above. Here the calyx bundles, carrying with them the petal midribs, turn outwards and upwards straightway. These bundles are shortly followed by those for the two staminal whorls. These relations, taken by themselves, would lead us to expect antepetalous loculi as in *L. Githago*. But another factor must be considered in the present case, viz. the size of



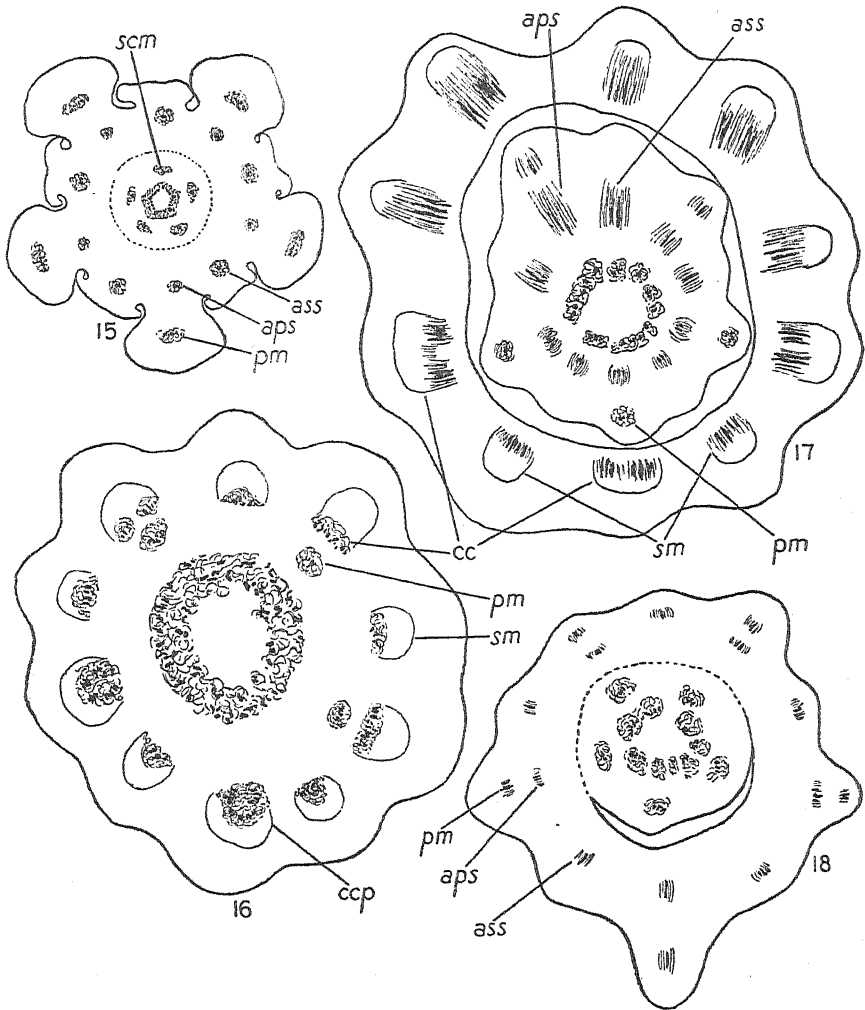
FIGS. 9-14. Caryophyllaceae. *Lychnis coeli-rosa*. Desr. (continued). All from transverse sections taken at successively higher levels except 14. 9. Ovary stipe. Towards the periphery the midrib bundles of the five antesepalous sterile carpels. In the centre the residual vascular elements which later become differentiated into the fertile carpel midribs and placental strands. The bundle  $x$  is about to be reabsorbed into the residual complex. 10. The same after differentiation of the fertile carpel midribs, which have turned outwards to form a single ring with those of the sterile carpels with which they alternate. Nearer the centre the placental strands (for explanation of the position of these strands see p. 246). In the centre three groups of unappropriated vascular elements. 11. Ovary base, slightly more developed towards the back than in front, showing some of the ten carpel midrib bundles turning outwards preparatory to the formation of the loculi. Nearer the centre placental strands and unappropriated vascular elements. 12. Ovule-bearing region of the ovary. In the outer wall the midrib bundles of the ten carpels. In the central area a ring of five compound bundles (for explanation of their position see p. 246), and an unappropriated central strand which later becomes incorporated with them. 13. Upper region of the ovary. In line with each fertile carpel is an area of conducting tissue beside which are the twin placental strands proper to each fertile member. Branches of these strands run out into the funicles. [For simplicity only the funicles of the ovules are represented.] 14. Diagram showing the origin of the trunk cords which give rise to the midribs (ascending) of the petals and the commissural bundles (descending) for the calyx.

the detached petal bundles, which are enormously large as compared with the corresponding bundles in the other species examined. With these bulky vascular cords blocking the way on this set of radii the exit of the antepetalous stamen bundles is delayed. Those for the antesepalous stamen members emerge before them. Then, there being no unusual conditions to interfere with the further development, the carpel bundles turn out in normal sequence. Hence the antesepalous members expand first and the loculi appear in a line with the sepals.

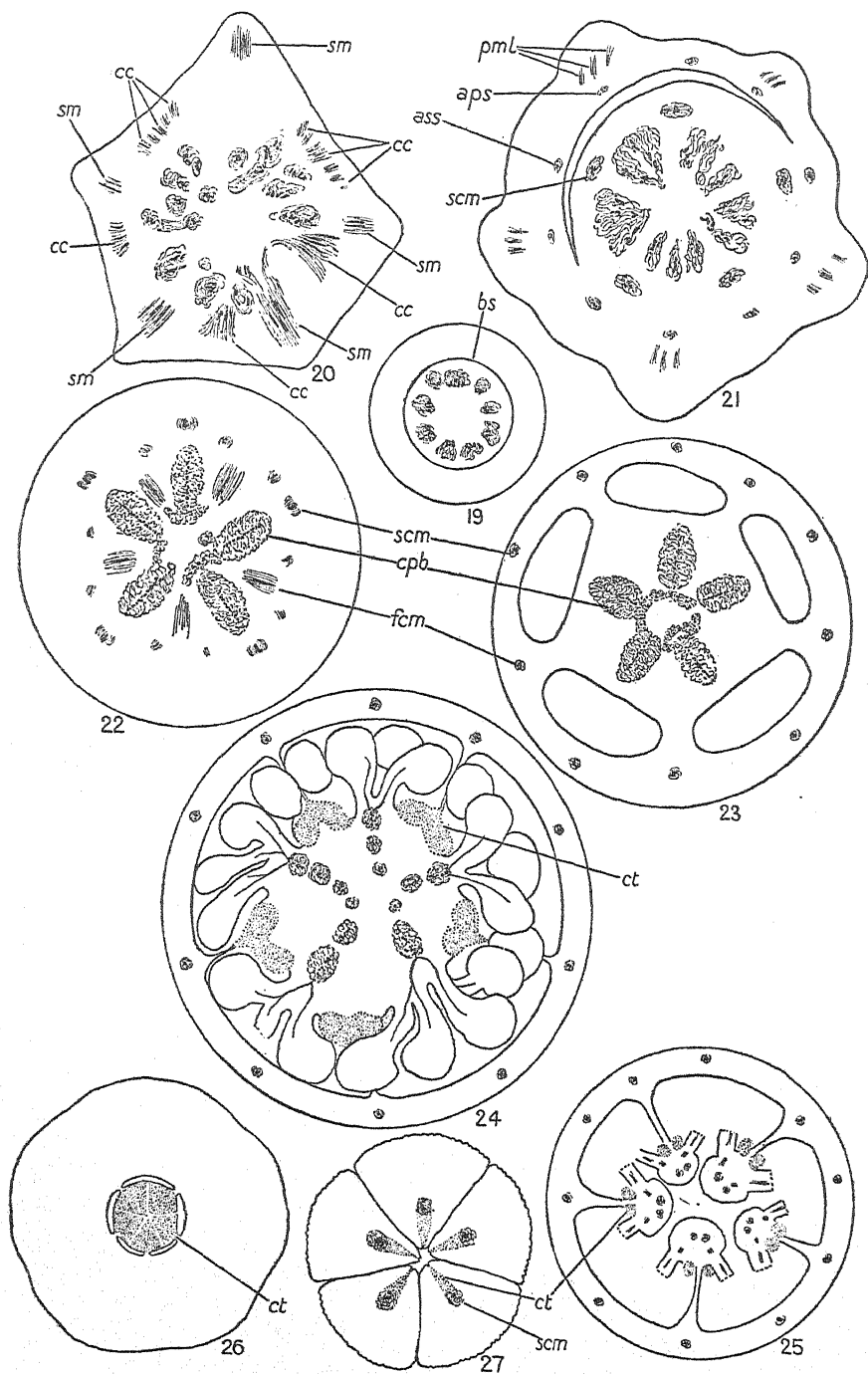
*L. flos-cuculi*, L., presents yet another set of conditions. Here a short but appreciable interval occurs between the exsertion levels of calyx and corolla. In this respect this species is thus intermediate between *L. (Agrostemma) Githago*, Scop., on the one hand, and types such as *L. coeli-rosa*, Desr., on the other. In correspondence with this intermediate condition the outer carpel midribs, as they arise, take up a position neither exactly in line with the sepals nor with the petals but midway between them. Such a disposition is readily intelligible on the present interpretation of carpel number and space relations, but on the older view it remains an unexplained anomaly.

From the foregoing discussion it will be clear that whereas on the traditional view that a single whorl of fertile valve carpels goes to the construction of the caryophyllaceous gynoecium we are unable to account for the non-occurrence of obdiplostemony in the isomerous types or for the varying position of the loculi, these features receive a rational explanation on the view that two carpel whorls are present which come to stand in a single ring, and that whether the antesepalous or the antepetalous whorl is the first to extend in the radial direction preparatory to the formation of the loculi, depends upon whether the level of exsertion of the calyx and the manner of origin of the vascular cords for the several outer whorls are such as to render the resistance to further radial extension greater on the radii of the petals or of the sepals.

A further feature of the ovary calling for explanation in many of the Silenoideae is the peculiar disposition of the vascular tissue in the placentae (see e.g. Figs. 12 and 23), though in view of the exceptional mode of placentation it is scarcely surprising that the placental strands should show a somewhat unusual arrangement. We get perhaps the clearest indication of placental relations in forms with a well-developed stipe such as *Silene pendula*, L. (Figs. 28–36). In this species it is plainly seen that when the three sterile carpel midribs take their rise, the whole vascular mass lying on the corresponding radii turns out from the central ring, leaving no placental strand behind but a clear ray of parenchyma continuous with the pith (Figs. 32, 33). As these bundles reach the periphery they undergo branching in the wall of the ovary in a pinnate manner characteristic of the valve carpel. On the other hand, when the midrib bundles of the three

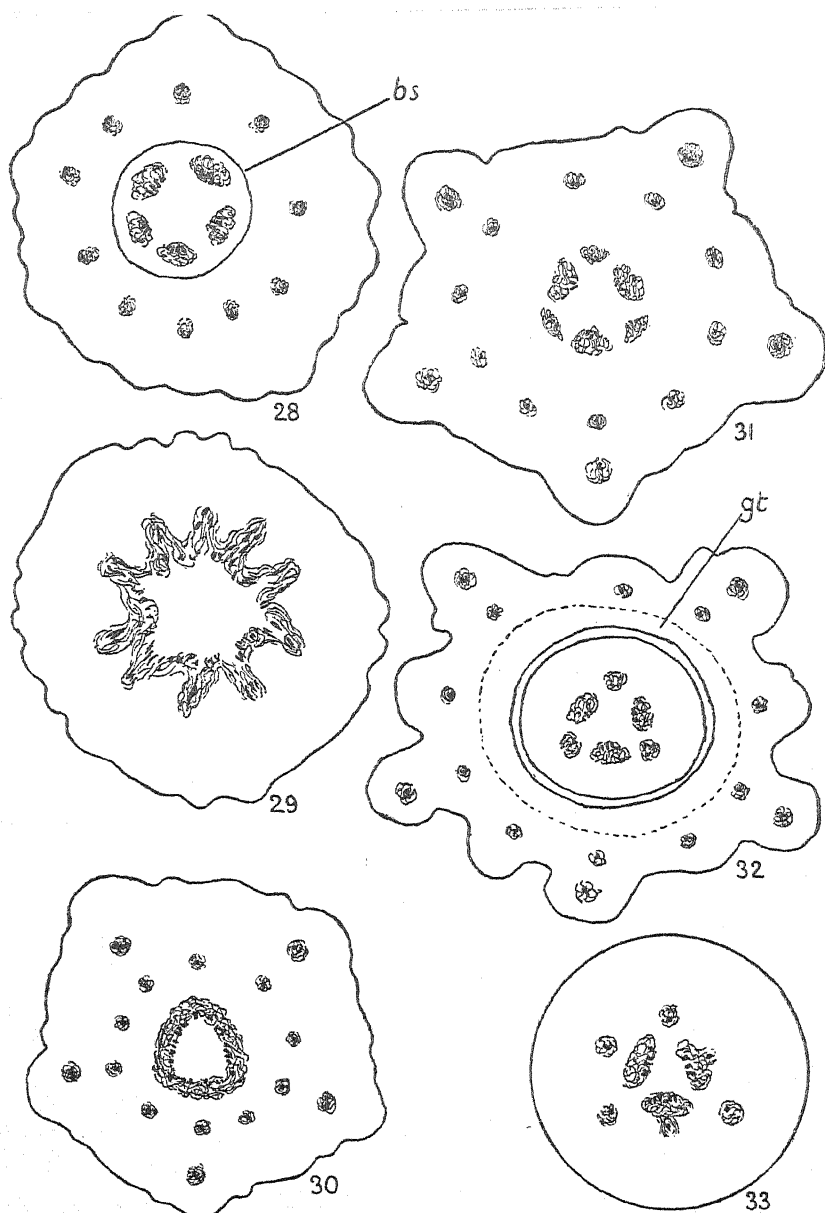


FIGS. 15-18. Caryophyllaceae (continued). 15. *Lychnis chalconica*, L. Flower base after exertion of the calyx, showing the corolla-stamen tube not yet disjoined from the gynoeceal tissue. The sterile carpel midribs are seen in line with the antesealous stamen bundles (see p. 244). 16-18. *Lychnis (Agrostemma) Githago*, Scop. from transverse sections taken at successively higher levels. 16. Flower base. Towards the periphery the five sepal midrib bundles alternating with the five trunk cords, which are in process of dividing up into the commissural bundles of the calyx and the petal midribs. In the centre the residual vascular ring serving androecium and gynoeceum. 17. The same showing the exerted calyx tube surrounding a central pentagon in which the petal bundles occupy the angles. Nearer the centre the ten stamen bundles which have just left the central ring. 18. The same at the level at which the corolla-stamen tube is becoming disjoined from the stipe. The bundles for the five sterile carpels have turned out from the residual vascular ring in line with the petals (see p. 244).





FIGS. 19-27. Caryophyllaceae (continued). *Lychnis dioica*, L. ♀. All from transverse sections taken at successively higher levels. 19. Flower stalk. 20. Flower base at the level at which the vascular bundles for the perianth are turning out from the central cylinder and those for the androecium (suppressed) are in process of differentiation. 21. The same at the level at which the stipe is becoming disjoined from the corolla tube in which the bundles for the ten stamens are present, although the stamens themselves do not take shape. In the stipe the sterile carpel midrib bundles have turned out from the central cylinder. [The exerted calyx is not shown]. 22. Ovary base before the appearance of the loculi showing towards the periphery the antesealous sterile carpel bundles; on the alternate radii and somewhat nearer the centre the main bundles of the antepetalous fertile carpels; in line with the sterile carpel midribs the vascular masses for the compound placentae are in process of differentiation. 23. The same after the appearance of the loculi. 24. Ovule-bearing region of the ovary. In the midline of each fertile carpel an area of conducting tissue abutting on the central parenchyma. On each alternate radius the radially extended placental complex from which the funicle strands arise as single or conjoined bundles. 25. Upper region of the ovary. The pith has now come to an end, leaving the inner face of the fertile carpels free so that the loculi become continuous. [For simplicity the body of the ovules is not represented.] 26. Ovary apex showing the five areas of conducting tissue now forming a central core. [The vascular tissue is not represented]. 27. The same at the level at which the sterile carpel midribs turn inwards to enter the styles. The areas of conducting tissue after becoming divided in half have re-formed into five new areas and are now situated on the other set of radii in line with the sterile carpel midribs. The vascular tissue of the fertile carpels has come to an end.



FIGS. 28-33. Caryophyllaceae (*continued*). *Silene pendula*, L. All from transverse sections taken at successively higher levels. 28. Middle region of gynophore showing the ten descending bundles for the sepal midribs and the commissural bundles of the calyx in a ring round the central vascular cylinder, which has a well-marked bundle sheath. (The exerted tube of the calyx is not shown.) 29. Top of gynophore where the five bundles for the sepal midribs and the five trunk cords for the commissural ribs and petal midribs leave the central cylinder. 30. Flower base showing petal and stamen bundles and the residual vascular cylinder now becoming three-sided. 31. The same after differentiation of the vascular masses for the six carpels but before the stipe is yet defined. 32. The same after corolla-stamen tube and stipe have become disjoined. A belt of glandular tissue (defined by dotted circle) lines the inner face of the tube. 33. Ovary stipe. The whole of the three vascular masses situated at the angles of the residual ring in 32 have turned out to form the sterile carpel midribs. On the alternate radii the midrib bundles for the fertile carpels are in process of following suit, but in this case leaving behind a large placental portion.

fertile carpels turn out on the alternate radii they leave behind in the centre a considerable part of the vascular mass from which they spring, and this residual portion supplies the strands to the funicles. But the exit of the midrib bundle has left each residual portion divided in half. The two parts not only for the time being remain distinct but diverge, each coming into contact, and merging with the adjacent half portion of the placenta of the neighbouring fertile carpel on that side. Hence at the level at which the loculi come into existence we see in cross section the six midrib bundles in the ovary wall and in the centre three placental bundle masses *in line with the loculi*. From their position and appearance at this level these masses might be taken to be unit bundles derived from the set of carpel midribs standing in line with them and the loculi. But in fact, as their origin shows, they represent in each case the vascular elements, in close juxtaposition, of two half placentae belonging to the adjacent carpels to right and left respectively, that is to say, to the carpels forming the septa. Only in the upper part of the ovule-bearing region, after reduction through supplying strands to numerous ovules, do the two component portions of each of these masses become distinct, and, reversing their earlier course, diverge, so that the twin halves of each whole placenta once again come to stand in their proper position, in line with the septum, i.e. with the carpel to which they belong. This final stage is best seen in those types in which the pith ceases just below the top of the placental column, so that the inner face of the fertile carpels becomes defined and free, thus leaving no doubt as to which of the placental bundles constitute the pair belonging to each fertile carpel. These relations of carpels and placentae are also clearly demonstrable in the ovary of *Cucubalus baccifer*, L. (Figs. 41–6), in which the vascular masses of the three placentae are separated from one another by parenchymatous tissue (Fig. 46). Again, in the dime-rous ovary of *Saponaria officinalis*, L. (Figs. 37–40), notwithstanding that the two placental vascular masses merge in the centre, the right and left position of the two constituent xylem bundles shows that they are properly assigned to the inner lateral pair of carpels (Fig. 37), while in the smaller ovaries of *Tunica saxifraga*, Scop., and *Tunica prolifera*, Scop., midribs and placental strands show clearly the same relations at the ovary base as those described above in *Silene pendula*.

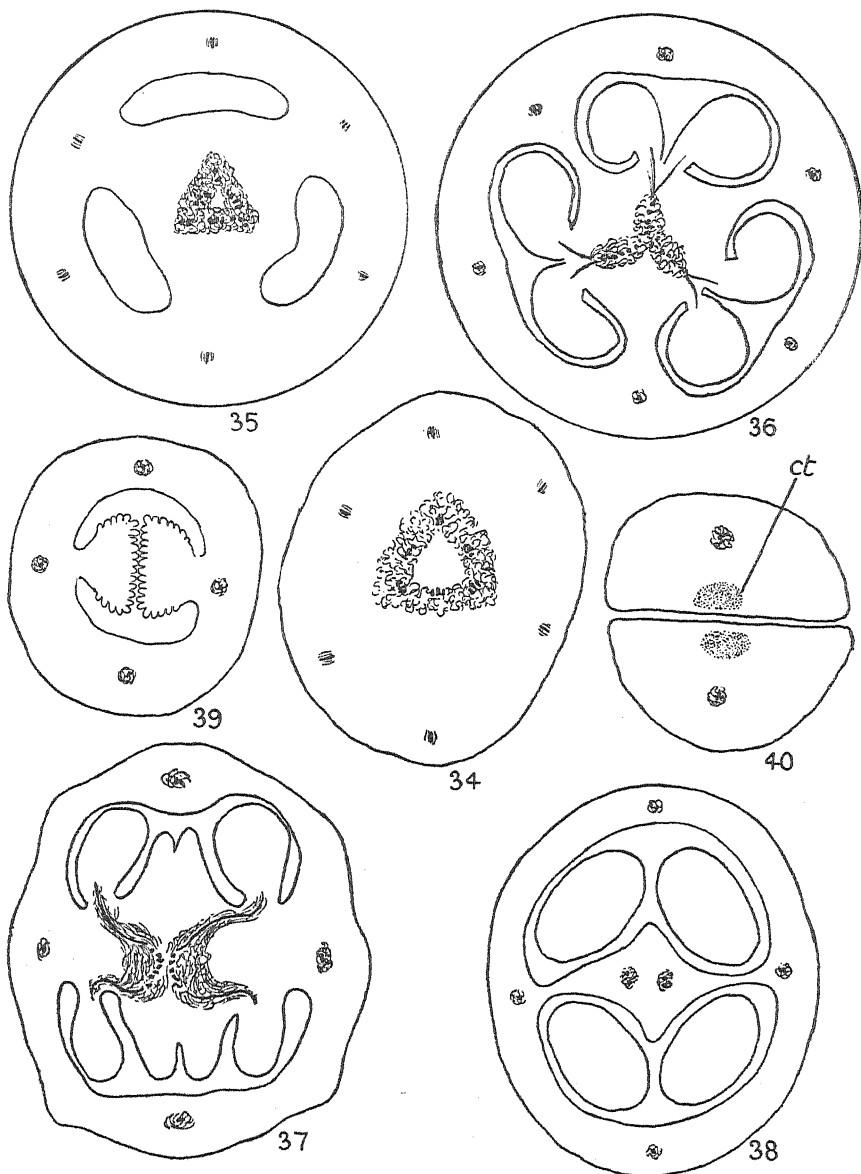
The two above-mentioned genera are included together with *Dianthus*, *Gypsophila*, and some few other genera in the subsection Diantheae, which is commonly described as differing from the Lychnideae in that it lacks the commissural bundles in the calyx. This account, though conveniently indicating a certain difference in external appearance, is not strictly accurate. In both *Dianthus* and *Saponaria* calyx commissural bundles arising in the usual way appear to be generally (? universally) present. They were also found in the two species examined of *Tunica* (*T. Saxifraga*, Scop., and

*T. prolifera*, Scop.). The true distinctive feature of these genera is not that these bundles are completely lacking, but that immediately upon their formation they divide up into their two components, and that these two components, which at once diverge, may fork again. Thus in these types the gamosepalous calyx always possesses more than the ten main parallel bundles (five midrib, five commissural) characterizing the Lychnideae. *Saponaria calabrica*, Guss., *Tunica Saxifraga*, Scop., and *Tunica prolifera*, Scop., for example, showed fifteen bundles at the level of exsertion. *Saponaria officinalis*, L., twenty-five (midribs with a pair of laterals and commissural pairs), *Dianthus superbus*, L., thirty-five (midribs with a pair of laterals and forked commissural pairs). In *Dianthus* and *Saponaria* the whole series of bundles are distributed more or less evenly round the whole circumference, but in *Tunica* the commissural pair proper to each sepal come to lie almost alongside the midrib, thus giving a false appearance that commissural bundles are entirely wanting. The allied genus *Gypsophila* is of particular interest in the present connexion, for in some species we meet with the same *apparent* absence of these bundles, while in others they are *genuinely missing*. In the case of the seven species examined they were found to be present in two (*G. elegans*, Bieb., and *G. perfoliata*, L.) and lacking in the other five (*G. fratenis*,<sup>1</sup> *G. libanotica*, Boiss., *G. paniculata*, L., *G. repens*, L., *G. transylvanica*, Spreng.). This same variability was observed also in certain genera of the Primulaceae (see later, p. 260). It would be a point of considerable interest to establish whether this character remains constant *within the species* or whether it varies with climate or other environmental conditions, the evidence available being too meagre to permit of any pronouncement at the moment.

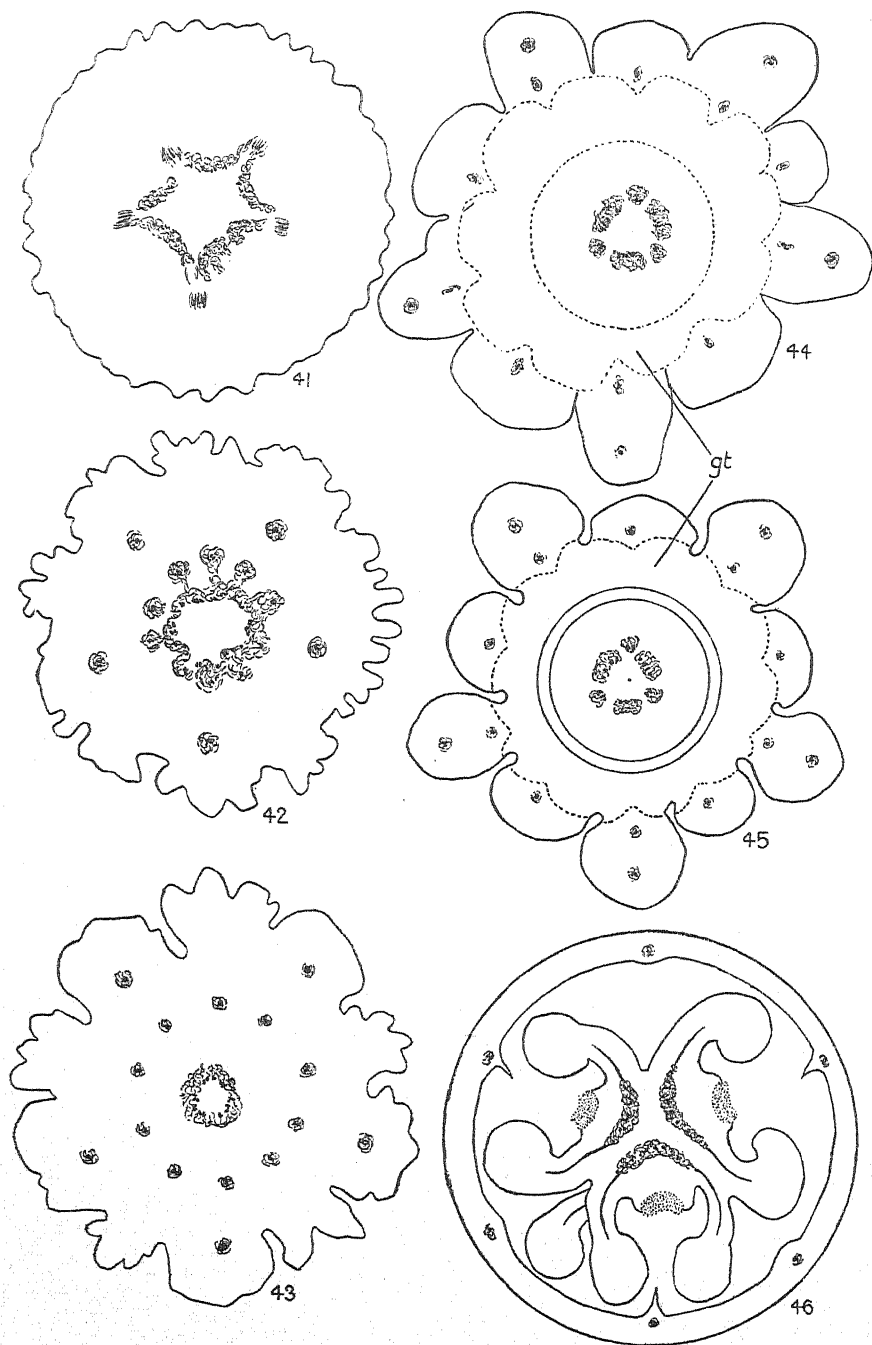
It remains to draw attention to the fresh light which the present interpretation of the ovary throws upon the variable manner in which the capsule splits at the apex, the number of teeth in some species being equal in number to the loculi and styles, and in other species as many again. Moreover in the former case dehiscence occurs in some types in line with the loculi and styles and in others on the alternate radii. This variability is independent of the number of carpels to the whorl, as may be seen in the diagrams illustrating fruit dehiscence given by Eichler (3), p. 114, Fig. 42. On the view that the ovary is composed of a single carpel whorl, this diversity remains a puzzling feature. On the present interpretation of the ovary as a 2-whorled structure in which the sequence, outer, sterile, valve, followed by inner, consolidated, fertile, is constant, but in which the starting-point of the sequence occurs on the one set of radii or the other according to varying limitations in respect of space (or time), such diversity presents no difficulty.

In the mature capsule the wall consists of a continuous sheet of

<sup>1</sup> A form listed by some seedsmen.



FIGS. 34-40. Caryophyllaceae (continued). All from transverse sections taken at successively higher levels. 34-36. *Silene pendula*, L. (continued). 34. Ovary base showing towards the periphery the six carpel midribs and in the centre, enclosing the pith, a three-sided figure from which the placental bundles are differentiated. 35. The same after the appearance of the loculi. 36. Ovule-bearing region of the ovary. The residual central triangle of vascular tissue seen in 34 and 35 has now become condensed into three compound placental bundles in line with the sterile carpel midribs. 37-40. *Saponaria officinalis*, L. 37. Middle region of the ovary showing the vascular elements supplying the two placentae as two separate masses with two laterally placed xylem bundles from which strands run to the ovules. 38. Upper region of the same showing the two placental bundles now drawn apart. 39. Ovary apex, above the ovule-bearing region but before the loculi become closed. The placental bundles have now come to an end. The pith having disappeared, the two fertile carpels are now separate along their inner face. 40. The same at the level of origin of the two styles which stand over the sterile carpels.



FIGS. 41-6. Caryophyllaceae (*continued*). *Cucubalus baccifer*, L. All from transverse sections taken at successively higher levels. 41. Flower base above the level of exertion of the calyx, just after the five bundles for the petals have become free. (The exerted calyx is not represented.) 42. The same at the level of origin of the stamen bundles. 43. The same after all ten stamen bundles have left the central ring, which is now becoming three-sided with xylem elements at scattered points. 44. The same after the stipe has become defined but not yet disjoined from the corolla-stamen tube, showing the bundles destined for the sterile carpel midribs at the angles of the three-sided figure and the vascular masses for the fertile carpels occupying the flat sides. 45. The same after disjunction. The vascular tissue as in the preceding figure. 46. Ovule-bearing region of the ovary. The placental bundles are now clearly seen in their true relation. (In 44 and 45 the irregular dotted outline defines the limit of a broad belt of glandular tissue lining the corolla-stamen tube.)

mechanical tissue in which the midrib bundles constitute so many lines of weakness down which splitting takes place for a longer or shorter distance. If fertile and sterile carpel midribs are both strongly developed splitting occurs down both sets of bundles, giving rise to twice as many teeth as there are styles and loculi as in *Lychnis dioica*, L., *L. diurna*, Sibth., *Dianthus*. If the two sets of midribs offer unequal resistance splitting is either confined to the one set or takes place more extensively down the one set than the other. Thus it comes about that in the pentamerous ovary, only five broad teeth arise which may remain entire or become slightly split, and which stand in line with loculi and styles (most species of *Lychnis*) or with placentae and septa (*L. viscaria*, L.) according as the fertile or the sterile carpel midribs split the more easily. In no case, therefore, does the individual tooth correspond with a whole carpel. In the first case mentioned above, each tooth is formed of two adjacent and conjoined halves of two neighbouring carpels ( $\frac{1}{2} \frac{1}{2}$ ) and hence is not superposed on a perianth member, but stands on an intermediate radius. In the other two cases the tooth being the termination of  $\frac{1}{2} + \frac{1}{2}$  carpels is centred over the whole carpel and in line with a perianth member.<sup>1</sup>

## 2. ALSINOIDEAE. Sepals free. (Figs. 47-56).

We find the same variability in the position of the loculi in isomerous genera of the Alsinoideae as in the Silenoideae. It is to be noted in this connexion that, although the sepals in this section are free, the vascular scheme of the calyx is in essentials the same as when they are conjoined. That is to say, both midrib and commissural bundles are formed in the Alsinoideae, the latter carrying out with them the bundles for the petals.<sup>2</sup> But other and new conditions have here to be taken into account in considering the causes affecting the position of the loculi. These are discussed below in the case of the three genera *Cerastium* (Figs. 47, 48), *Spergula* (Figs. 49, 50), *Sagina* (Fig. 51), which may be taken as illustrative types. In none of the three is a gynophore present, yet the loculi occur in different positions, being antesepalous in *Cerastium*, antepetalous in *Spergula* and *Sagina*.

In the species of *Cerastium* examined (*C. arvense*, L., *C. chloraefolium*, Fisch. and Mey., *C. alpinum*, L.) the ten calyx bundles carry out with them not only the bundles for the petals but also for a short distance those for the androecium (Fig. 47). Hence here there will not, presumably, be the same degree of resistance to the radial extension of the valve carpels as is met with in those types where (as in the Silenoideae described above) *four*,

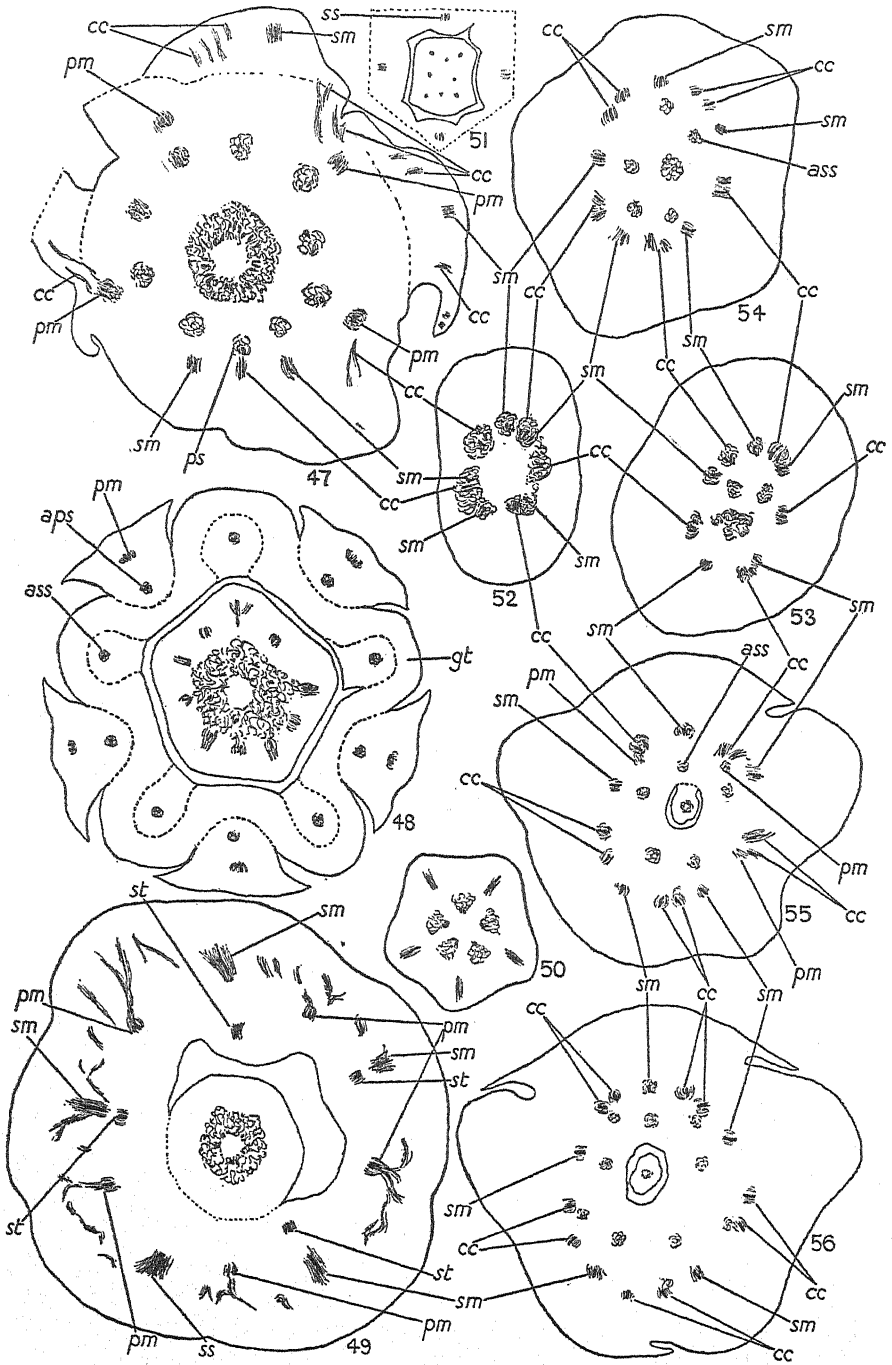
<sup>1</sup> Except in such forms as *Lychnis flos-cuculi* as explained above (p. 246).

<sup>2</sup> This mode of formation of the marginal veins of the sepals has been found to be of widespread occurrence even in the polysepalous calyx. It is characteristic, for example, in the Cruciferae. It is not, however, possible to deal at length with this point in the present account. It is proposed to treat it in greater detail in a separate communication.



*successive* whorls of bundles have previously turned out from the central cylinder. As a result the valve carpel midribs, which in these species turn outwards before the almost negligible stipe has become disjoined from the perianth tube, arise in proper alternating succession, and the loculi appear in line with the sepals (Fig. 48). As in the Silenoideae, the valve carpel midribs comprise all the vascular elements on these radii, the vascular elements for the placentae being left behind by the fertile carpel midribs when they turn out from the centre.

In *Spergula* (*S. arvensis*, L., Figs. 49, 50) and *Sagina* (*S. procumbens*, L., Fig. 51, and *S. nodosa*, Fenzl) the mode of origin of the bundles for the floral whorls is somewhat different, taking place in a manner which appears to be characteristic of many of the small-flowered genera included in this section (*Polycarpon*, *Polycarpaea* (Figs. 52–6), *Spergularia*, *Arenaria Illecebrum*). These types resemble *Cerastium* in so far that the vascular bundles for corolla and androecium are carried out with the ten trunk cords which furnish the midribs and commissural bundles of the sepals (Fig. 49). But these trunk cords do not, as in *Cerastium*, at once give off the stamen bundles and then continue their outward course. Instead, they pass intact to their furthest outward point before giving off inwards, on the one set of radii the bundles for the antesepalous stamens, and on the other set the trunk cords for petals and antepetalous stamens (if the latter members are present). Thus whereas, in *Cerastium*, the course of the disjoined bundles for corolla and androecium is at first away from the centre, that of these bundles in *Spergula* and *Sagina* is centripetal. In both these latter genera there is an appreciable stipe (Figs. 50, 51). As flower tube and stipe become disjoined, the thickness of the tube on the radius of each sepal with its superposed stamen is seen to be greater than that on the alternate radii, the pressure of the bulging antesepalous stamens causing the stipe to become flattened on these radii, so that it appears pentagonal in cross section in *Spergula* (Figs. 49, 50), more or less square in *Sagina* (Fig. 51), with the angles in both cases in line with the petals. The external contour of the stipe is reflected in the outline of the vascular figure, which is 5-sided in *Spergula*, 4-sided in *Sagina*. As the level of expansion of the carpels is reached, bundles which become the valve carpel midribs turn out from the angles of the vascular cylinder, which lie in line with the petals. These bundles comprise all the vascular elements situated on this set of radii (see Fig. 50), so that we again have clear evidence that it is not the outer carpels from which the placental strands take their rise, and that the interpretation given above of the appearance seen in many placentae of the Silenoideae is correct. In both genera the centripetal extension of calyx and androecium causes greater resistance to the centrifugal radial expansion of the carpels on the radii of the sepals (as is indicated by the outline of the stipe) than on that of the petals, hence the valve carpel midribs and



FIGS. 47-56. Caryophyllaceae (*continued*). All from transverse sections taken, when in series, at successively higher levels. 47, 48. *Cerastium chloraefolium*, Fisch. and Mey. Flower base after perianth and stamen cords have been differentiated. In the centre the five-sided residual ring with the angles in line with the sepals. (Portions of one petal and two sepals have been cut away above on the left.) 48. The corolla-stamen tube, with glandular ring, surrounding the ovary stipe in which the sterile valve carpel midribs are seen turning out in line with the antesealous stamens. 49, 50. *Spergula arvensis*, L. 49. Corolla-stamen tube and stipe in process of becoming disjoined. Residual vascular tissue of the stipe forming a five-sided figure with the angles in line with the petals. 50. Ovary stipe showing the sterile valve carpel midribs turning out from the angles of the vascular pentagon. 51. *Sagina procumbens*, L. Ovary stipe showing form assumed through compression by the perianth-stamen tube. 52-6. *Polycarpha Teneriffae*, Lam. 52. Flower stalk. In the residual vascular cylinder the xylem elements are already arranged alternately in single and paired strands preparatory to the formation of the trunk cords for sepal midribs and antesealous stamens (with single xylem strand), and those for the commissural bundles of the calyx and petal midribs (with double xylem strand). 53. Flower base showing the expanded vascular cylinder breaking up into the trunk cords indicated in 52. Undifferentiated vascular elements derived from the inner border of the cylinder, which provide the bundles for the gynoeceum, are seen converging towards the centre. 54. The same after the trunk cords on the sepal radii have split up into the sepal midribs and the antesealous stamen bundles. 55. The same at the level at which flower tube and stipe have become partially disjoined. The vascular elements for the petal midribs are beginning to separate from the commissural bundles of the calyx. 56. The same showing flower tube and stipe completely disjoined.

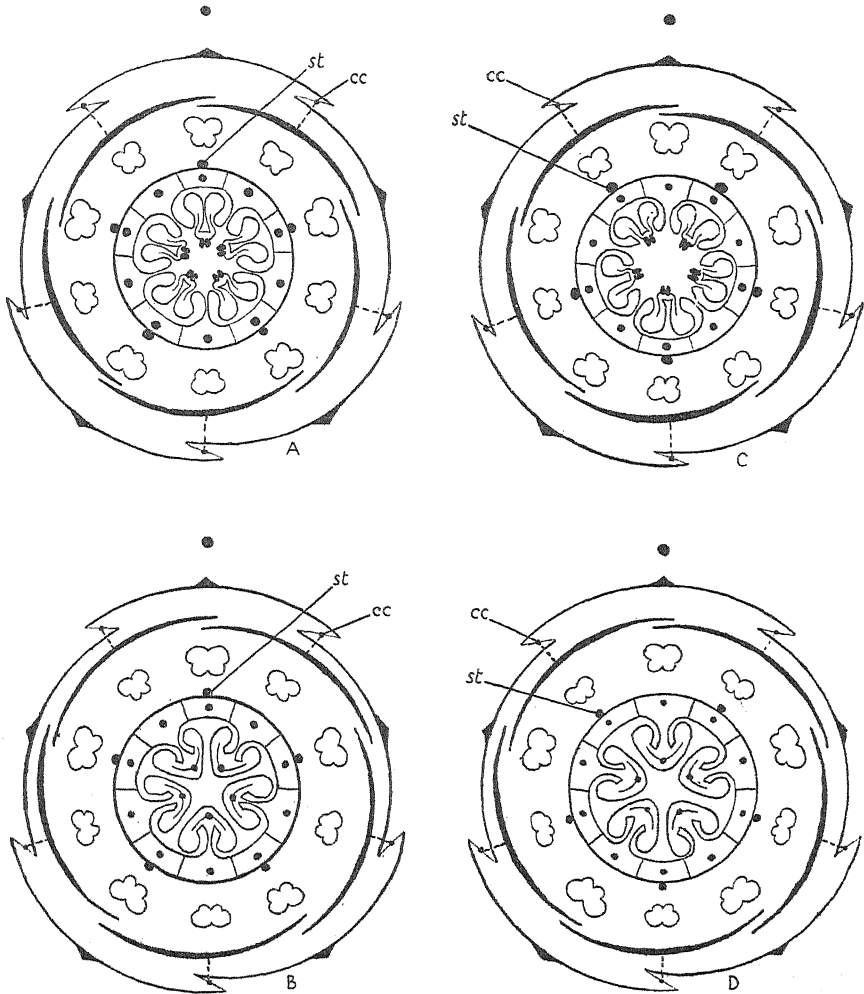
the loculi are antepetalous. Both genera have suffered the loss of the fertile carpel midribs, all the differentiated vascular elements on these radii remaining in the centre and passing entire into the placentae. It is apparent that in these alsinoid types, which lack a gynophore and in which a preliminary expansion of the vascular cylinder at the flower base results in the appearance of a single peripheral trunk cord on each radius, which gives rise in succession *from without inwards* to the bundles for perianth, and stamen whorls, resistance to carpel expansion comes into play, producing the same effect as described above for *Lychnis* (*Agrostemma*) *Githago* (an isomerous silenoid also without a gynophore), although the bundles for the androecium in this latter species take their rise direct from the residual cylinder, proceeding *from the centre outwards*. In other words, the effects of space restriction come into play, whether the carpels have to expand when still surrounded by, and continuous with, the tissues of the perianth and stamen whorls for which two cords have previously turned out in succession from the central cylinder on each radius; or whether, although already disjoined from the flower tube, for which only one trunk cord has been required on each radius, they nevertheless become subject to compression owing to the increase in the radial dimension of the outer whorls taking place in a centripetal direction.

#### PRIMULACEAE. (Figs. 57–88 and Diagrams E–I).

In the Primulaceae, where again we meet with 'free-central' placentation, we find as well some of the features demanding explanation which have been discussed in the above account of the Caryophyllaceae. These puzzling features are found on investigation to arise here from the same cause and to be explicable in the same way. This being so, it will be advisable here also to deal briefly with certain features of the outer whorls before turning our attention to a detailed consideration of the gynoecium.

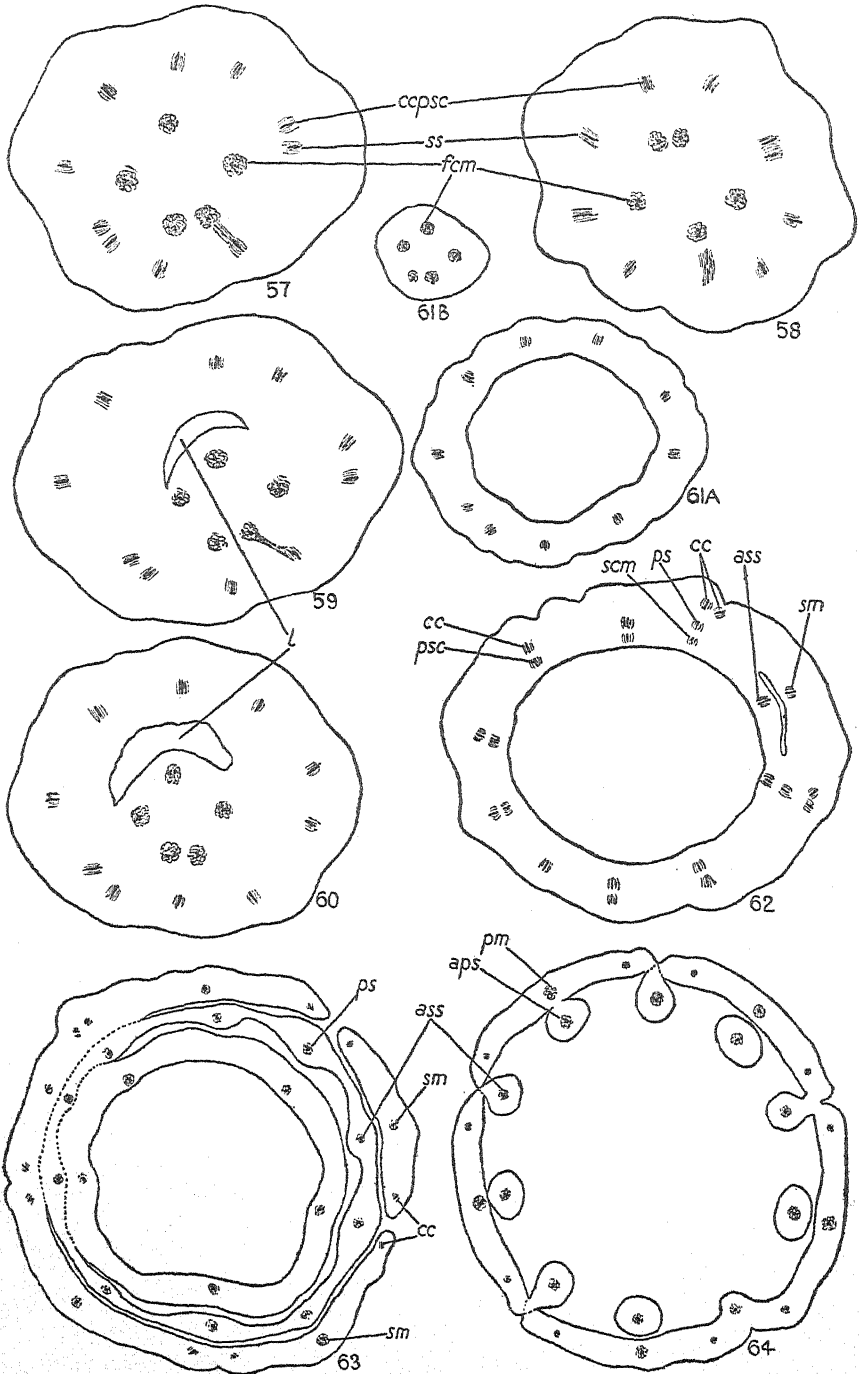
*Perianth.* The calyx varies in that it may show only the five main bundles of the sepal midribs, or it may be 10-ribbed with five additional, commissural bundles on the alternate radii. The presence or absence of these commissural bundles appears generally to be a constant feature for the species, but both conditions are often to be found within the genus. Thus commissural bundles are present in *Primula auricula*, L., *P. glaucescens*, Moretti, *P. malacoides*, Franch., *P. marginata*, Curt., *P. obconica*, Hance, *P. saxatilis*, Kom., *P. Veitchii*, Duthie, *P. verticillata*, Forsk.; in *Androsace primuloides*, Duby, *A. sarmentosa*, Wall., and vars. *Chumbyi* and *yunnanensis*, *A. sempervivoides*, Jacquem. and Duby, *A. villosa*, L. type (Fig. 83) and var. *arachnoidea*; in *Anagallis arvensis*, L. (Fig. 68) *Coris monspeliensis*, L., *Lysimachia barystachys*, Bunge, *L. nummularia*

L., *L. pseudo-Henryi*, Pampan., *L. punctata*, L., *L. quadrifolia*, L., *Steironema ciliatum*, Rafin., *Samolus repens*, Pers., (Fig. 63) *Soldanella alpina*, L.



A-D. Caryophyllaceae. A, B. *Lychnis coeli-rosa*, Desr. Type with gynophore, loculi antepetalous. A, showing the gynoeceum at a lower, B, at a higher level. In A false pairs of placental strands stand in line with the loculi; in B the pair of placental strands proper to each fertile carpel is shown as a single vascular cord alternating with the loculi. C, D. *Lychnis (Agrostemma) Githago*, Scop. Type without gynophore. Loculi antepetalous, otherwise as in *L. coeli-rosa*. c.c., calyx commissural bundles; st., style.

They were found on the other hand to be lacking in *Primula acaulis*, Hill, and various derivatives, *P. elatior*, Jacq., *P. veris*, Lehm., Garden Polyanthus, *P. Bulleyana*, Forrest, *P. capitata*, Hook., *P. chrysopa*, Balf. f. and Forrest, *P. Cockburniana*, Hemsl., *P. Columnae*, Ten., *P. darialica*, Rupr., *P. denticulata*, Sm., *P. farinosa*, L., *P. floribunda*, Wall., *P. frondosa*,



FIGS. 57-64. Primulaceae. *Samolus repens*, Pers. All from transverse sections taken at successively higher levels from the same flower except 58. 57. Flower base. The enlarged vascular ring consists of the ten trunk cords for the ten perianth members, superposed stamens and staminodes, and five sterile carpels. Nearer the centre the five bundles for the five fertile carpels in process of detachment from these cords. 58. The same stage from another flower. 59. The same at the level at which the fertile carpel column begins to separate from the enclosing ring consisting of perianth, androecium, and outer carpels, still conjoined and undifferentiated. 60. The same after the last fertile carpel cord has become independent. 61A. The flower wall. 61B. The now completely disjoined fertile carpel column. 62. The flower wall showing stages in the breaking up of the trunk cords on the one set of radii into the sepal midribs and staminode bundles, and on the other set of radii into the calyx commissural bundles, petal-stamen trunk cords, and sterile carpel midribs. On the right a split in the tissue where the calyx and corolla whorls are becoming disjoined. The adjacent commissural bundle at either end of the split has divided in two, preparatory to becoming in each case the marginal veins of neighbouring sepals. 63. The same at the level at which the sepals are becoming free and the petal-stamen tube dissociated from the ovary wall. The sterile carpel midribs stand in line with the petals. On the right one sepal now completely free. 64. Corolla tube with the ten members of the androecium in process of becoming detached.

Janka, *P. hirsuta*, All., *P. Menziesiana*, Balf. f., *P. pubescens*, Jacq.; in *Androsace carnea* v. *brigantiaca*, Jord. and Fourr. (Fig. 79), *A. coronopifolia*, Andr., *A. lactea*, L., *A. lactiflora*, Fisch., *A. obtusifolia*, All., *A. pyrenaica*, Lam.; in *Asterolinon stellatum*, Hoffm. and Link., *Douglasia vitaliana*, Benth. and Hook., *Soldanella montana*, Willd., *Lysimachia clethroides*, Duby, *L. Fortunei*, Maxim. Some, but not all, of the commissural bundles were observed in *Primula anisodora*, Balf. f. and Forrest, *P. florindae*, Ward, and *Androsace chamaejasme*, Willd.

When the commissural bundles are present they turn out from the central cylinder carrying with them the petal midribs as in the Caryophyllaceae. This union, which usually continues for an appreciable distance, but exceptionally is resolved immediately, is best seen in types in which the bundles for the perianth whorls run horizontally for some distance as they leave the central cylinder; in those in which the vascular cylinder itself expands at the level of origin of the sepal bundles so that the horizontal course of these bundles is thereby shortened, the association is less apparent. Here, as in the Caryophyllaceae, it may be inferred that the vascular elements which in a 5-ribbed calyx would form part of an issuing sepal midrib bundle, to be given off later (where branching occurs) as lateral veins, in the 10-ribbed types become detached prematurely and, diverging to right and left, become conjoined with the petal midribs. Exceptionally the calyx may show fifteen bundles at the level of exsertion as in *Primula Palinuri*, Petagn. This condition arises through the separation, at the moment at which each trunk cord becomes dissociated into its calyx and corolla components, of the two halves composing the calyx portion of the cord. These halves immediately diverge constituting *separate* marginal veins for the sepal to right and left to which, on this interpretation, they truly belong.

*Androecium.* Except in *Samolus* and *Soldanella* and in *Lysimachia thyrsiflora*, L., and *Steironema ciliatum*, Rafin., there is usually no external trace of the suppressed antesepalous stamen whorl. Nevertheless, the corresponding vascular bundles are generally present, and were in fact observed in every type investigated.<sup>1</sup> They are invariably carried out from the central cylinder conjoined with the sepal midribs, becoming disjoined later, just as the antepetalous stamen bundles are at first combined with the petal midribs and later become detached. After dissociation these bundles come to stand at about the same distance from the centre as the petal-stamen trunk cords on the alternate radii, so that later, when calyx and corolla are successively exserted, the detached antesepalous stamen bundles, in the absence of the corresponding members, pass up in the corolla tube.

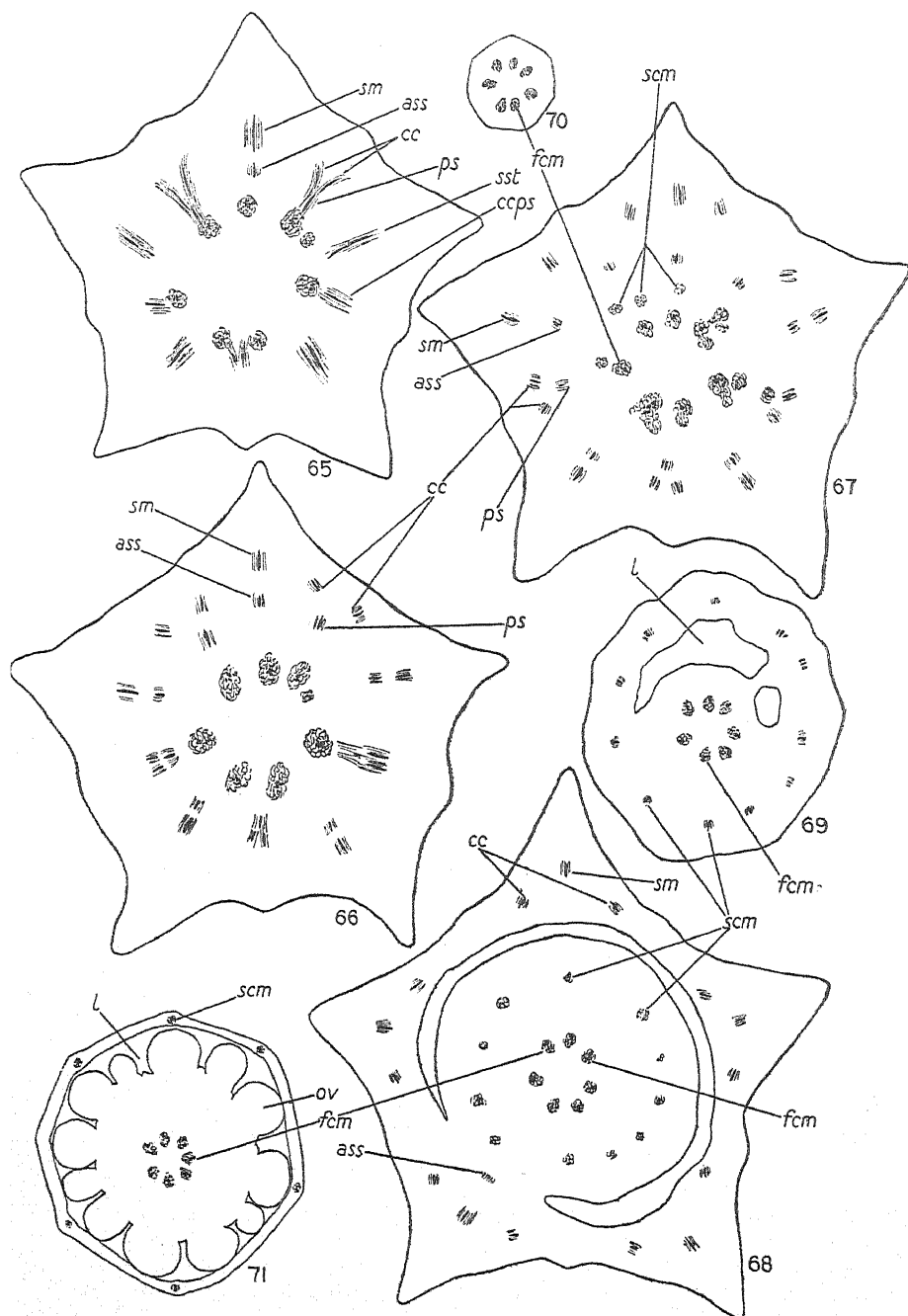
<sup>1</sup> Though some of the five bundles were lacking in the flowers examined of *Androsace chamaejasme*, Willd., *A. coronopifolia*, Andr., and *A. lactea*, L., and in some specimens of Garden Polyanthus (Fig. 73); in other specimens of the last-named form all the bundles were missing.



This is the case also in *Steironema ciliatum* and *Lysimachia thyrsiflora* where, although vestiges of the antesepalous stamens in the form of teeth or scales are still present, the bundles no longer enter these structures. But in *Samolus*, where the antesepalous stamen members develop as distinct full-sized structures (Fig. 64), each of the corresponding five bundles branches into three strands, the central one entering the staminode, and each lateral strand the petal on the corresponding side. That the persisting bundles of the antesepalous stamen whorl, when that whorl fails to attain any considerable morphological expression, should run upward in the tube of the gamopetalous corolla is the natural outcome of their being carried out conjoined with the sepal midribs. Indeed, this association, in the circumstances, leaves no alternative, if these stamen bundles are not to be left outside the closed ring of the corolla tube, which would involve a reversal of the normal sequence of perianth and stamens. We may, in fact, expect that these relations will hold good generally, so that in any family exhibiting the conditions described above we shall be prepared to find that the corolla tube may show a vascular bundle on the radii between the petals as well as in the midline of the petals, just as we have learned to expect to meet with the condition of obdiplostemony in any 6-whorled, hypogynous, dicotyledon type if the bundles for the two stamen whorls turn out from the centre *separately from those for the two perianth whorls*, unless some structural modification affords the necessary relief from congestion in some other way.

It will now be clear from this account of the origin and course of the bundles destined for the androecium that the superposition of the one existing staminal whorl on the petals—the subject of so much discussion in the past—involves no real difficulty. For here, as elsewhere, *alternation of successive whorls is founded on the alternate emergence of the vascular cords which supply these whorls*. Hence, in the Primulaceae, where perianth-androecium trunk cords are formed on all ten radii, the alternate arrangement will not be between *successive individual* whorls—sepals, petals, outer stamens (if present), inner stamens, but between calyx  $\pm$  superposed stamens behaving as one whorl and corolla  $\pm$  superposed stamens behaving as the succeeding whorl. This principle has been previously demonstrated in many cases, an outstanding example being afforded by *Pyrus*, in which the position of the carpels always remains constant despite the variations in the number of staminal whorls, since the bundles for the whole androecium are carried out conjoined with those for the perianth (8). In like manner the position of the outer carpel whorl in the Primulaceae is unaffected by the presence or absence of the outer staminal whorl.

*Gynoecium.* Some account of the primulaceous gynoecium was given, it may be recalled, at an earlier stage of the present investigation (7, p. 152, Fig. 66, and p. 154). It was there shown that the theory of carpel polymorphism provides a satisfactory solution of the various difficulties



FIGS. 65-71. Primulaceae (*continued*). *Anagallis arvensis*, L. All from transverse sections taken at successively higher levels from the same flower except 71. 65. Flower base. In line with each angle of the pentagon a trunk cord composed of a sepal midrib and the persisting bundle of the suppressed staminode seen cut longitudinally. The posterior cord has already become resolved into its two components. On the alternate radii calyx commissural bundles are becoming detached from the petal-stamen cords. Residual masses which will later differentiate into the bundles for the ovary wall and ovule-bearing column are seen around the pith in process of becoming detached from some of the above-mentioned trunk cords. 66. The same showing the bundles, now more or less independent, for sepals (midribs and commissures), petals, and both staminal whorls. Nearer the centre an inner ring of bundles for the gynoecium. 67. The same showing the sterile carpel midrib bundles in process of turning out from the residual vascular ring. 68. Calyx tube (with midrib and commissural bundles) and ovary almost disjoined. (The petal-stamen tube has fallen away except on the lower left side, where all the whorls remain conjoined and one antesealous stamen bundle is still to be seen.) 69. The ovary showing partial separation of the ring of eleven (?) conjoined sterile carpels from the column of seven fertile carpels. 70. The inner carpel column now completely free. 71. The ovule-bearing region of the ovary from another specimen, which also has seven fertile carpels but only six sterile members.

presented by the gynoeceum in this family and that in the typical case, when the full ground-plan is realized,  $G = 10 + 10$ . In view of the numerous points requiring consideration, it will be well, however, to deal here with the whole question in somewhat greater detail. As the need for such detailed investigation becomes apparent as soon as a survey of genera and species is undertaken, one cannot but feel considerable surprise at the statement of Pax (6), p. 115, that the gynoeceum in *Primula* is really simpler to explain than the androeceum. For, as shown above (pp. 264-7), a study of the vascular anatomy at once makes clear that the relations of the androeceum are perfectly simple, whereas in the gynoeceum there are many unusual features requiring elucidation.

According to the old interpretation, the ovary wall is regarded as consisting typically of five antesepalous carpels of the valve type, from which the edges have become detached; while the central ovuliferous column is supposed to be formed of these detached portions which include the marginal veins. Now, it must be realized that no single piece of positive evidence from the normal flower can be adduced in support of this conception. It is common knowledge that the ovary wall arises as an even, unbroken ring, that there are no septa, and that the single style filament ends usually, if not invariably, in an undivided stigma. Hence these features, often of considerable value as indicators of the carpel number, in the present case afford no clue. When we come to examine the vascular anatomy, we find the same lack of confirmation of the interpretation that  $G = 5$ . In certain types, it is true, the ovary wall may show five equidistant vascular bundles, but this condition is frequently found to hold only for the individual flower or plant, not for the species as a whole. Moreover, when five such midrib bundles alone are found, they stand in some types in line with the sepals, in others in line with the petals. Much more frequently the number of such main bundles exceeds five, varying from six to ten or more. *Still more difficult to reconcile with the traditional conception is the frequent total want of any uniform numerical relation between the number of supposed midrib bundles in the ovary wall and the number of separate and independent fertile strands presumed to represent marginal veins, which pass up into the ovuliferous column.* Indeed, the supposition that  $G = 5$  appears to rest on no more solid foundation (as was appreciated by Eichler (2)) than mere analogy with the pentamerous plan of the outer whorls, and inference, drawn from the number of teeth or valves in the ripe capsule. But the number of these teeth varies between five and ten, and when five in number they may be superposed either on the petals or on the sepals. Hence, even in regard to this feature certain assumptions must be made in order to 'square' the facts with theory, such as that dehiscence may occur down the midribs, or down the supposed commissures, or in both positions.

In certain abnormal flowers of various species of *Primula* (*acaulis*,

*veris*, *sinensis*, *japonica*) and of *Anagallis arvensis*, the ovary wall takes on the form of five leaf-like structures. In one such recorded instance in *P. veris* each of these five structures bore a style and stigma (5). Such cases have been considered to be due to disjunction of the carpels (dialysis), and thus to afford confirmation of the traditional interpretation. Eichler, who accepted the current view, presumed that such separate members would naturally stand on the radii of the sepals (2, p. 327, footnote \*\*). He concludes that Celakovsky's reference to an *antepetalous* position of such separate members (1, p. 170) is probably an oversight, since this author in another context (p. 171) describes them as being *antesepalous*. Furthermore, on the ground that his figures do not bear out his statement that the separate carpel members were antepetalous in similar cases occurring in *Anagallis arvensis*, Eichler also throws doubt on the accuracy of the observations of Marchand (4). But, as the present account makes clear, Eichler's own premise cannot be accepted. When only five carpels go to the formation of the ovary wall they are situated, as stated above, in some species in line with the sepals and in others in line with the petals, hence if dialysis should occur the separate members will stand on a different set of radii in the two cases. But apart from this point it is difficult to conceive in either alternative that carpels which are regarded as having reverted more or less to the form of foliage leaves should still be supposed to suffer detachment of their margins, for an ovuliferous column is present in these abnormal specimens as in the normal ovary. So difficult, indeed, does the conception appear that the facts might more logically be held to disprove, than to support, the traditional explanation of the nature of the column. And as regards the separate leaf-like structures themselves—is it altogether certain that they correspond to individual carpels? and that the abnormality is one of simple dialysis? I have not, unfortunately, been able to obtain ovaries, of this abnormal type for examination, and in those accounts in which figures are given, details regarding the vascular anatomy are generally lacking. It must be borne in mind that in the species of *Primula*, in which these abnormalities have usually been observed, the number of carpels forming the ovary wall (according to the present interpretation) is generally ten (or more), as shown by the ten (or more) primary bundles. It would, therefore, seem probable that we are not dealing in these cases with simple disjunction of the several carpel members, and that the superficial resemblance of the five structures to individual leaves is as illusory here as the similar appearance has been shown to be in the classic instance of *Sterculia (Firmiana) platanifolia*, L. (11). In this latter type a premature longitudinal splitting in the midline of the fertile carpels gives rise to five leaf-like structures. Each of these structures, however, is not the equivalent of a *single individual leaf*, but corresponds to  $\frac{1}{2}1\frac{1}{2}$  carpellary leaves. The same type of abnormality is by no means rare among the Cruciferae, where median

longitudinal splitting of the two fertile carpels results in the formation of two separate valve-like structures. These leaf-like structures are not, as I have previously pointed out (9, p. 619), the counterparts of the two valves of a normal siliqua, for they are not formed merely of the two sterile valve carpels but of these carpels bordered on each side by half of the intervening consolidated fertile carpel.<sup>1</sup> That is to say, each represents  $\frac{1}{2}1\frac{1}{2}$  carpels. Now of the ten bundles occurring in many cases in the wall of the normal primulaceous ovary, one set of five is usually more strongly developed than the other set, and is often alone continued up into the style filament. This inequality supplies the condition which would permit of the same kind of happening here as in the ovary of *Sterculia*, and I strongly suspect that a median split of one set of five carpels is precisely what takes place in primulaceous types when the ovary wall takes on the form of five leaf-like structures. Furthermore, this interpretation enables us to understand how it could come about that the products of such splitting should stand in some species in line with the sepals and in others in line with the petals, should this diversity of position be definitely confirmed, as may well be the case.

We may now more fully consider the features of the normal gynoecium from the standpoint that it consists of *two* carpel whorls,<sup>2</sup> the outer, sterile whorl forming the ovary wall and style filament, the inner, fertile whorl, the ovuliferous column.

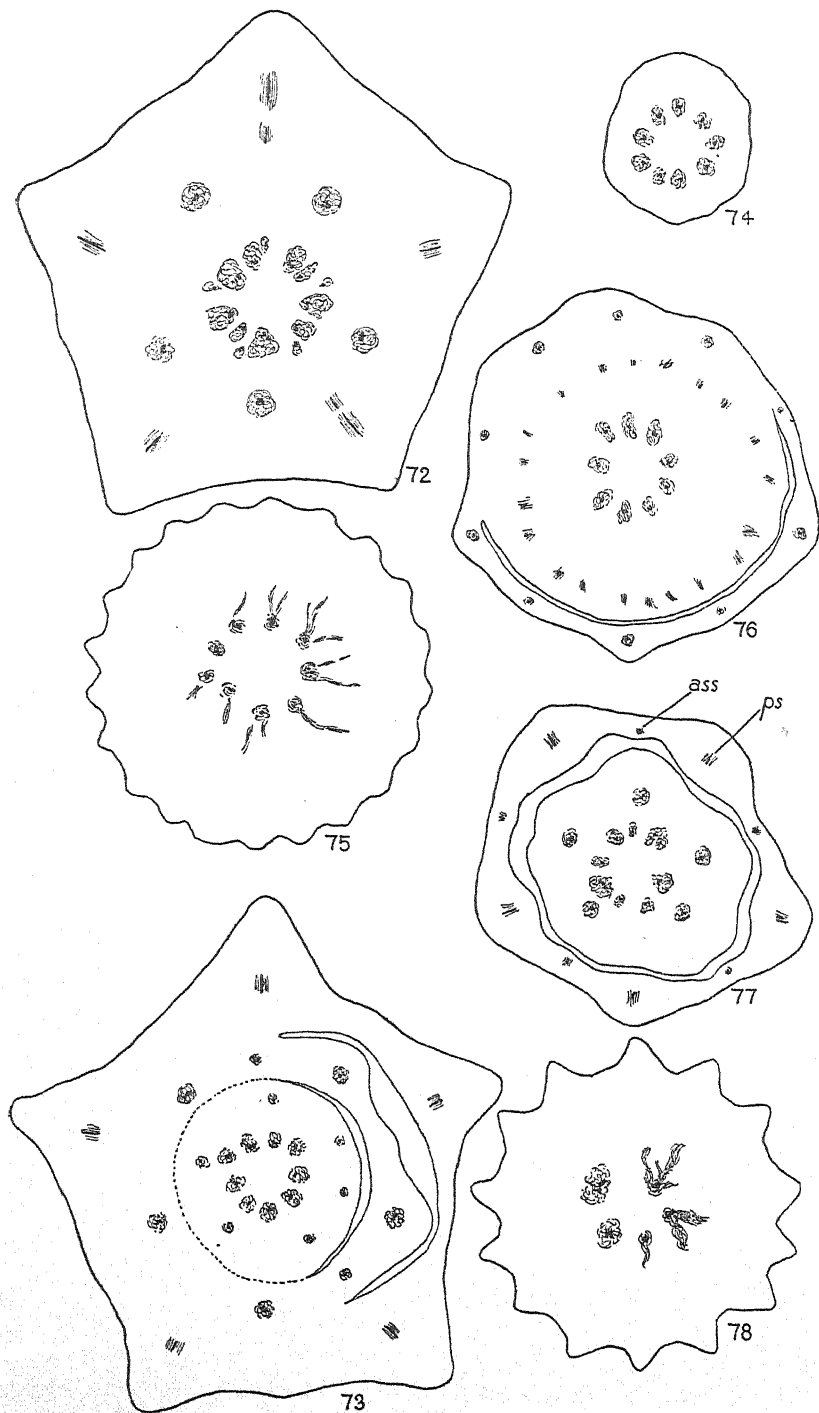
*The ovary wall.* Differentiation of the vascular strands passing up, in the ovary wall generally proceeds in one of two ways. It must be borne in mind that it is on the radial disposition of these strands that we have in the main to rely for an indication of the number of carpels present. In the one case the strands in question turn outwards from the residual vascular cylinder; in the other, bundles given off from the inner side of the enlarged vascular ring turn inwards towards the centre. In the simplest cases, as when five strands turn outwards on the radii of one perianth whorl, we may safely conclude that five carpels are present. Thus five antesealous carpels appear to be characteristic in *Primula capitata*, Hook., *P. chrysopa*, Balf. f. and Forrest, *P. dariatica*, Rupr., *P. denticulata*, Sm., *P. farinosa*, L., *P. floribunda*, Wall. (Fig. 77), *P. frondosa*, Janka. More rarely, when only five bundles are formed, they may stand in line with the petals. This was observed in some flowers of *Anagallis arvensis*, L., although in this genus

<sup>1</sup> I had at one time thought it possible that such splitting of the siliqua might also come about through the arrested development of the fertile members, but I have come to doubt whether the split immature siliqua ever arises from this cause.

<sup>2</sup> Strictly speaking, no doubt, when the full number of carpels (10 + 10) is present the ring both of the sterile and of the fertile carpels is composed each of two secondary pentamerous whorls. But, as the members of each ring generally arise *simultaneously* and come to stand in a single circle, it appeared less confusing to refer to each individual ring as one whorl than to describe it as composed of two whorls, a terminology which would suggest a *successive* development comparable with that of calyx and corolla which is not, in general, to be made out.

higher numbers are more general. Equally regular and symmetrical is the development of five antepetalous carpel bundles in species of *Lysimachia* (*L. nummularia*, L., *L. pseudo-Henryi*, Pampan., *L. punctata*, L., *L. quadrifolia*, L.), in *Steironema ciliatum*, Rafin., and in *Asterolinon stellatum*, Hoffm. and Link. In all the above cases the five carpel midribs usually remain unbranched and continue up into the style filament. The question arises as to the cause of this variability in position of the five carpels in types having the same floral formula ( $K_5 C_5 A_5$ ). [The vestiges of the second androecium whorl which are present in *Steironema* in the form of teeth may be ignored from the present point of view, since they are non-vascular.] In seeking for the explanation one turns naturally to another morphological character, in respect of which the Primulaceae fall into two classes, viz. those with, and those without, calyx commissural bundles. Since the development of such commissural bundles may be supposed to involve the premature emergence of the petal-stamen trunk cords in this family just as the presence of these same bundles has been shown to entail the early departure of the petal bundles in the Caryophyllaceae,<sup>1</sup> we might expect that the outermost carpel whorl in the Primulaceae would be antepetalous or antesepalous according as these calyx bundles are present or absent. Such a connexion here is, however, not easy to establish. In the first place, wholly new conditions affecting spatial relations must obtain in many primulaceous types. As previously stated, in some forms the whole vascular cylinder expands at the flower base. From this expanded ring the perianth-androecium trunk cords turn horizontally outwards, while those for one or both carpel whorls turn horizontally inwards. Here conditions affecting the spatial relations of these whorls will not necessarily be the same as when the sterile carpel bundles follow centrifugally upon those for the outer whorls. In other species in which the bundles for the sterile carpels as well as those for the calyx and corolla, on the other hand, turn out successively from a central vascular ring, the number of these sterile carpel bundles may lie between five and ten, or may even exceed ten. In these cases there can clearly be no uniform correspondence of the carpel radii with the radii of the perianth members. Other types again must be excluded from consideration, either for the reason that the sterile carpel bundles do not arise separately but are conjoined for a longer or shorter distance with those of the petals (*Samolus*, *Asterolinon*) or because, although arising separately, they do not leave the central cylinder until the level is reached at which perianth and ovary are becoming disjoined (*Douglasia*), when again the conditions set up will not necessarily be similar to those obtaining in types where the whorls

<sup>1</sup> Though in the Caryophyllaceae the natural outcome of the presence of these commissural bundles is only seen in types like *Lychnis* (*Agrostemma*) *Githago*, where the effect is not counter-balanced and outweighed by the development of a gynophore or by other complications.





FIGS. 72-8. Primulaceae (*continued*). All from transverse sections taken, when in series, from below upwards. 72-5. Garden Polyanthus (G 6 + 9). 72. Flower base. At the angles of the pentagon the bundles for the five sepal members. In this specimen only two of these bundles gave off the strand for the suppressed stamen member. On the alternate radii the five petal-stamen trunk cords. In the centre the residual vascular ring serving the gynoeceium from which five small bundles in line with the sepals are about to emerge to become the midribs of five sterile carpels. 73. The same at the level at which calyx and corolla are becoming exerted. (The flower is now further developed on the one side than on the other.) In addition to the five antesealous sterile carpel bundles seen in the preceding figure, one antepetalous bundle has now emerged from the residual ring. In the centre an inner ring of nine vascular strands which become the main bundles of nine consolidated fertile carpels. 74. The ovuliferous column of nine carpels now free from the ovary wall which is not shown. 75. The same, now greatly enlarged, at the level of origin of the strands for the ovules. 76. *Primula sinensis*, Lindl. Flower base just above the level of exertion of the calyx, which is not shown. The corolla is in process of disjunction from the ovary, which shows an outer ring of numerous strands irregularly distributed destined for the ovary wall. In the centre the bundles of nine fertile carpels. 77, 78. *P. floribunda*, Wall. 77. The corolla tube and ovary now disjoined. In the ring of the corolla the five petal-stamen trunk cords alternating with five antesealous stamen bundles. Towards the outer boundary of the ovary the bundles for five antesealous sterile carpels. Nearer the centre the residual vascular tissue for the fertile carpels. 78. The ovuliferous column of five fertile carpels at the level of origin of some of the ovule strands.

immediately outside the gynoeceium are not yet exerted. But even though we rule out of consideration all cases coming under any of these heads and restrict comparison to those in which the sterile carpel bundles, like those of the preceding whorls, are five in number and turn out separately from a central cylinder, we are still confronted with the difficulty encountered in all Primulaceae, *irrespective of the presence or absence of calyx commissural bundles*, viz. the difference in vigour of development on the two sets of radii as evidenced by the unequal development of the two staminal whorls. The decline in vigour on the sepal radii appears frequently to extend beyond the androeceium and to involve the gynoeceium. For in many types showing at first ten outer carpel bundles the five in line with the sepals sooner or later die out, leaving only those in line with the petals to continue up the ovary wall into the style filament (species of *Primula*, *Androsace*, *Anagallis*). Hence it becomes difficult to determine whether in these circumstances an antepetalous position of the outer carpel bundles, such as was observed in some flowers of *Anagallis*, is due solely to the presence of calyx commissural bundles (which occur here) or whether this position is due entirely, or in part, to this inherent weakness on the other set of radii. It is, however, a significant fact that in the seven above-mentioned species of *Primula* (p. 270) which, this weakness on the sepal radii notwithstanding, regularly form five antesepalous carpels, these commissural bundles are lacking, whereas they are present in the several species of *Lysimachia* cited above and in *Steironema ciliatum*, in which the five carpels are antepetalous. From these facts it appears reasonable to conclude that unless additional disturbing factors come into play the sterile carpel whorl, if isomerous with the other whorls, follows the ordinary law of alternation and is antesepalous; but that where calyx commissural bundles are present the normal sequence of the next succeeding whorl having a separate origin is upset here as in other analogous cases, with the result that it is the antepetalous carpels which form the ovary wall.

Before leaving consideration of types having only five sterile carpels, brief reference must be made to the case of *Samolus* (Figs. 57–64), which also shows the combination of calyx commissural bundles with antepetalous sterile carpels. Although this disposition is in accord with the interrelations described above, it seems probable that in this genus weakness on the sepal radii rather than the presence of the commissural bundles may be the determining cause of the carpel position. For, owing to the fact that the flower here is syngonous,<sup>1</sup> the vascular bundles for the sterile carpels arise in a manner quite different from that in which they originate in all other members of the family so far investigated. At the base of the flower the central vascular cylinder expands; ten vascular bundles become differentiated, and from these ten trunk cords turn outwards. Five of these

<sup>1</sup> Used in place of the old misleading term 'epigynous'.

cords become dissociated later into two bundles, the outer one becoming a sepal midrib, the inner one passing in part into the superposed staminode just below the level of exertion, and in part into the corolla.<sup>1</sup> The other five alternate trunk cords break up in each case into three strands, a calyx commissural bundle towards the outside, and, in succession towards the inside, a petal-stamen cord and a sterile carpal midrib. Here it seems doubtful whether the formation of the commissural bundles can affect the carpal position in the same way as in the hypogynous types. On the other hand, it may well be that the decline in vigour which has brought about the sterilization of the antesealous members of the androecium has led further to the non-disjunction of carpellary strands on these radii, with resulting suppression of the set of antesealous sterile carpal members, and the appearance of the five persisting members on the radii of the petals.

In forms with more than five sterile carpels any number may occur from six to ten, or more. When the typical full complement of ten is present, as in individual flowers of various species (see list, p. 277) the ten midrib bundles may turn outwards from the central cylinder, one bundle occurring on the radius of each perianth member. Or, if this condition occurs in a form with an enlarged vascular cylinder, a single strand (or group of strands)<sup>2</sup> arising in line with each perianth member turns inwards, as was observed in *Primula cortusoides*, L. When intermediate numbers occur the bundles are often complete on one set of radii and incomplete on the other, and are then irregularly spaced. In these various cases it is usually an easy matter to ascertain the number of carpels present. But in other conditions this is difficult, if not impossible, to decide with certainty, owing either to the unequal vigour of the issuing bundles or to the formation of a large number of strands standing in no definite relation to the perianth radii, or to a combination of both conditions. When recourse can no longer be had to position in relation to the two sets of radii occupied by the calyx and corolla, it becomes practically impossible to distinguish between cases of early branching of the midrib bundles and those where two neighbouring midrib bundles turn out together and then separate. This difficulty was met with in *Primula sinensis* (Fig. 76), in various species belonging to the *auricula* section of the genus, and sometimes in *Anagallis*. The whole body of evidence taken together indicates that ten is typically the full number of sterile carpels, but that in many cases only those on one set of radii develop; that all intermediate stages occur, and that in some forms the normal full number is exceeded. In the majority of types only five of the bundles are prolonged into the style filament, but this is not invariable, as many as thirteen having been observed in a specimen of

<sup>1</sup> See above, p. 265.

<sup>2</sup> Owing to branching.

*Primula Forbesii*, Franch. to extend to the base of the style filament or even further, and eleven in a flower of *P. Columnae*, Ten.

*The ovuliferous column.* A study of the manner of origin of the bundles passing up into the ovuliferous column brings to light the fact—wholly at variance with the traditional conception of these bundles as representing prematurely detached marginal veins—that they do not arise in any orderly or definite manner, nor do they show any constant numerical relation to the sterile carpel bundles. This is equally the case whether the sterile carpel bundles turn *outwards*, in which case the individual bundles remaining behind usually (though not invariably) become differentiated directly into the main bundles of what are here regarded as fertile carpels, or whether the fertile bundles as they arise from the enlarged vascular ring turn *inwards*. This latter method of origin is well seen in *Samolus*, in which the enlarged vascular ring breaks up almost simultaneously into the ten trunk cords for the calyx, corolla, androecium and ovary wall,<sup>1</sup> and the five or six fertile bundles which turn inwards. Now here, if anywhere, we should expect, if the fertile bundles do, in reality, correspond to marginal veins, that they would in their origin show some fixed relation to the trunk cords on the one or the other set of radii, but instead they appear to arise from indeterminate points.

Having come into being, the fertile bundles in all types pursue an upward parallel course, coming to an end as they give off the strands to the ovules, many or few according to the species. The number of these parallel bundles, easily observed as a rule, may be taken to indicate the number of fertile carpels present. Difficulty arises only when for some cause the spacing becomes irregular and the interval between two neighbouring bundles is reduced to vanishing point. In such cases two xylem strands can still generally be distinguished. It then becomes rather an academic question whether the two corresponding carpels can be said still to exist or whether vascular elements belonging (phylogenetically) to two neighbouring carpels have now come to be utilized in the formation of one carpel. It follows that the number of fertile carpels is most readily observed where the number of bundles is small and the development of parenchyma considerable, since in these circumstances mergence of two neighbouring bundles is less likely to occur.

The accompanying list of the number of sterile and fertile carpels judged to be present in the flowers which were examined of various species will serve to illustrate the lack of numerical relation referred to above.<sup>2</sup>

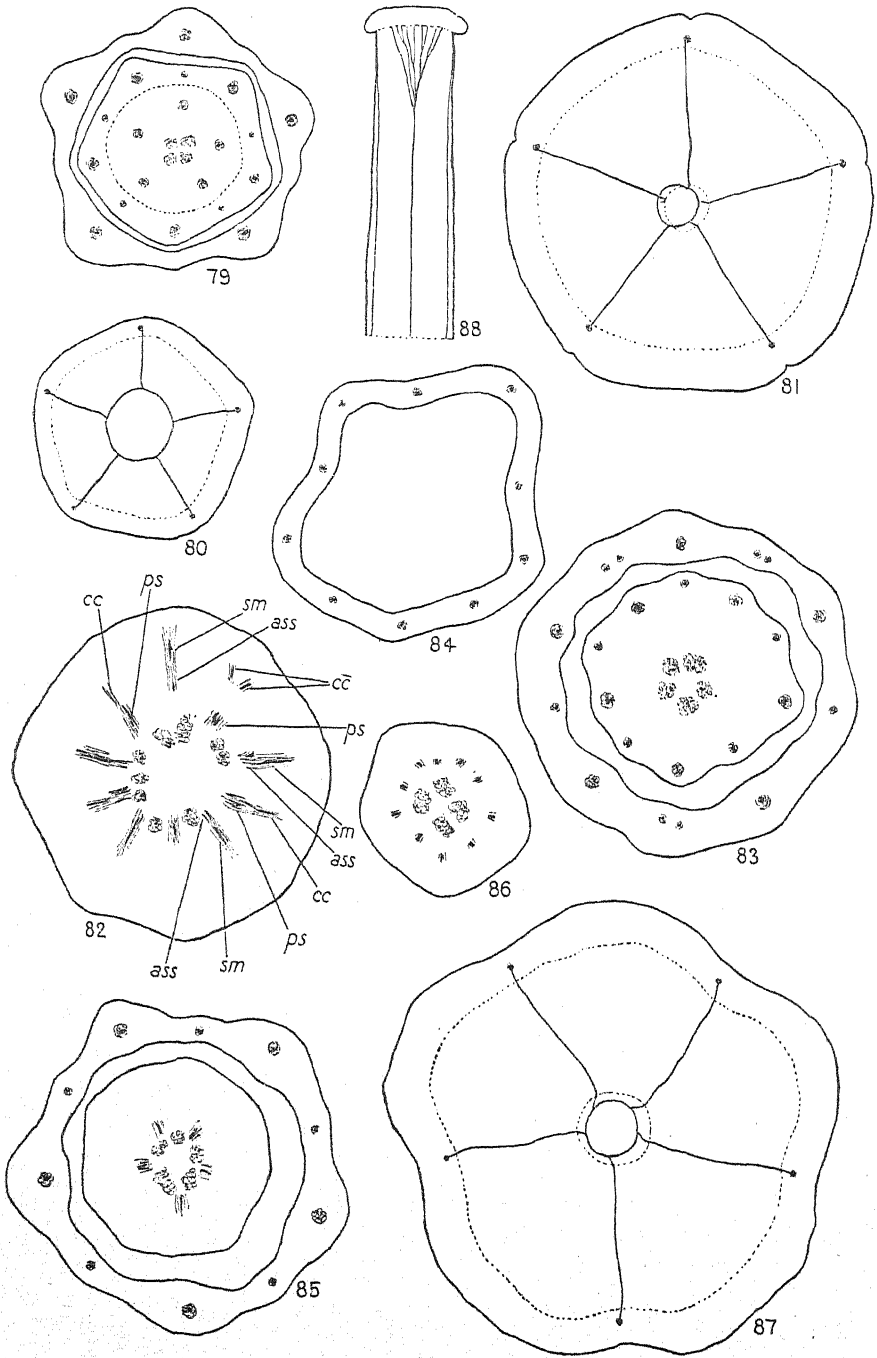
One further point remains to be considered, viz. the orientation of the

<sup>1</sup> See above, p. 274.

<sup>2</sup> It is not intended to convey that these records necessarily indicate the most characteristic numbers for each particular species. For such a determination a considerable number of counts made from time to time during the season would be necessary in each case.

Number of carpels judged to be present in individual flowers of various species of *Primula* and of other primulaceous genera.

Species.	Carpels.		Species.	Carpels.		Species.	Carpels.	
	Sterile.	Fertile.		Sterile.	Fertile.		Sterile.	Fertile.
<i>Primula acutis</i> , Hill.	> 10	5	Garden <i>Polyanthus</i> .	10	10	<i>Asterolinum stellatum</i> , Hoffing. and Link.	10	5
"	> 10	7	<i>Primula geraniifolia</i> , Hook.	> 6	7	"	5	4
"	> 10	9	<i>P. glaucescens</i> , Moretti	> 10	> 10	<i>Asterolinum stellatum</i> (tetramerous flower)	5	3
"	> 10	10	<i>P. hirsuta</i> , All.	> 10	> 10	<i>Asterolinum stellatum</i> (hexamerous flower)	4	4
" blue derivative	9	6	<i>P. japonica</i> , A. Gray	> 10	6	<i>Cortusa monspeliensis</i> , L.	6	3
<i>P. anisodora</i> , Balf. f. and Forrest.	> 10	5	<i>P. malacoides</i> , Franch	> 10	7	<i>Cortusa Mathioli</i> , L.	5	4
<i>P. auriculata</i> , L.	> 10	> 10	"	> 10	9	<i>Cyclamen persicum</i>	5	4
<i>P. Bulleyana</i> , Forrest	> 10	9	"	> 10	9	"	5	8
"	> 10	10	"	> 10	9	<i>Dodecatheon tetrandrum</i> , Suksdorf.	> 10	10
<i>P. capitata</i> , Hook.	> 10	3	<i>P. marginata</i> , Curt.	> 10	9	<i>Do glasia Vitaliana</i> , Benth. and Hook.	> 10	9
<i>P. chrysopa</i> , Balf. f. and Forrest.	5	6	"	> 10	6	<i>Lysimachia barystachys</i> , Bunge	10	?
<i>P. Cockburniana</i> , Hemsl.	5	8	<i>P. Menziesiana</i> , Balf. f.	> 10	10	"	8	7
<i>P. Columnnae</i> , Ten.	> 10	9	<i>P. obconica</i> , Hance.	> 10	10	<i>L. chelthroides</i> , Duby	9	8
<i>P. cortusoides</i> , L.	? 10	7	"	> 10	10	<i>L. Ephemenum</i> , L.	5	4
<i>P. darwinica</i> , Rupr.	5	5	<i>P. Palmieri</i> , Petagn	> 10	9	<i>L. Fortunei</i> , Maxim.	5	4
"	5	6	<i>P. pubescens</i> , Jacq.	10	8	<i>L. nummularia</i> , L.	10	3
"	5	5	<i>P. saxatilis</i> , Kom.	> 10	8	"	5	4
<i>P. denticulata</i> , Sm.	5	8	<i>P. sinensis</i> , Lindl.	> 10	8	<i>L. pseudo-Henryi</i> , Pampan. (hexamerous flower)	5	6
"	5	9	<i>P. Veitchii</i> , Duthie	> 10	6	"	6	8
<i>P. elatior</i> , Jacq.	5	10	<i>P. veris</i> , Lehm.	> 10	7	<i>L. punctata</i> , L.	5	> 7
<i>P. farinosa</i> , L.	> 10	6	<i>P. verticillata</i> , Forsk.	9	> 10	"	5	8
<i>P. floribunda</i> , Wall.	5	5	<i>Anagallis arvensis</i> , L.	6	6	"	5	8
"	5	5	"	6	6	"	5	8
<i>P. Florindae</i> , Ward	5	5	"	6	6	<i>L. quadrifolia</i> , L.	5	9
<i>P. Forrestii</i> , Franch.	10	7	"	6	6	<i>L. vulgaris</i> .	5	8
<i>P. Forrestii</i> , Balf. f.	> 10	5	"	7	6	<i>Samolus repens</i> , Pers.	10	6
<i>P. frondosa</i> , Janka	5	5	"	7	7	"	5	5
"	5	5	"	10	5	"	5	5
Garden <i>Polyanthus</i>	7	9	"	10	8	<i>Soldanella alpina</i> , L.	> 10	5
"	9	9	"	10	7	<i>S. montana</i> , Willd.	> 10	7
"	10	7	<i>Androsace lactea</i> , L.	> 10	7	<i>Stairionema ciliatum</i> , Rafn.	> 10	6
"	10	9	<i>A. lactiflora</i> , Fisch.	> 10	7	"	5	8
"	10	9	"	> 10	7	"	5	6



FIGS. 79-88. Primulaceae (*continued*). From transverse sections taken, when in series, from below upwards, except 88. 79-81. *Androsace carnea* v. *brigantiaca*, Jord. and Fourr. 79. Flower base. At the periphery the calyx, already exerted, with midrib but without commissural bundles. Within this ring the corolla tube, defined but not yet disjoined from the ovary. In the corolla tube the larger petal-stamen trunk cords alternate with the smaller antesepalous staminal bundles. Nearer the centre the bundles for the five antesepalous sterile carpels forming the ovary wall surrounding the four bundles for as many fertile carpels. 80, 81. The ovary after disjunction from the corolla, halved transversely, then rendered transparent and pressed flat. The two halves are viewed from inside. 80. The lower half. The midrib bundles of the five sterile carpels are seen passing out from the centre and turning upwards in the ovary wall. The innermost circle indicates the outline of the ovuliferous column, details of which are not shown. 81. The upper half. The five sterile carpel bundles are seen passing from the ovary wall into the style base. 82-83. *Androsace villosa*, L. 82. Flower base at the level at which the ten trunk cords for perianth and androecium turn outwards, leaving behind irregular masses which differentiate into the bundles for the carpel whorls. The cords on the one set of radii are in process of differentiation into the sepal midrib and the superposed staminal bundle, those on the alternate radii into the calyx commissural bundle (which at once bifurcates) and the petal-stamen cord. 83. The same after exertion of the calyx. To the outside the ring of the calyx in which the sepal midribs alternate with the divided or still undivided commissural bundles. Within the calyx ring an undifferentiated core of corolla and ovary tissue showing towards the periphery the petal-stamen cords alternating with the antesepalous staminal bundles. In the centre five undifferentiated bundle masses which serve the sterile and fertile carpel whorls. 84. The ovary wall, composed in this case of ten sterile carpels. 85, 86. Intermediate stages from another specimen. 85. The corolla tube and ovary now disjoined. In the corolla tube the five petal-stamen trunk cords alternate with the five antesepalous staminal bundles. The ovary is somewhat exceptional in that the bundles for the five antepetalous sterile carpels are differentiated appreciably earlier than those for the five antesepalous members. 86. Ovary base below the level of the loculus, showing ten bundles for a corresponding number of sterile carpels, and in the centre four vascular masses for a corresponding number of fertile carpels. 87. Upper half of the ovary wall rendered transparent and pressed flat. The five antepetalous carpel bundles alone persist to the top of the ovary and enter the style filament. (Compare with Fig. 81 where the persisting carpel bundles are antesepalous.) 88. The style filament rendered transparent, showing three of the five persisting carpel bundles, which branch copiously immediately below the stigma.

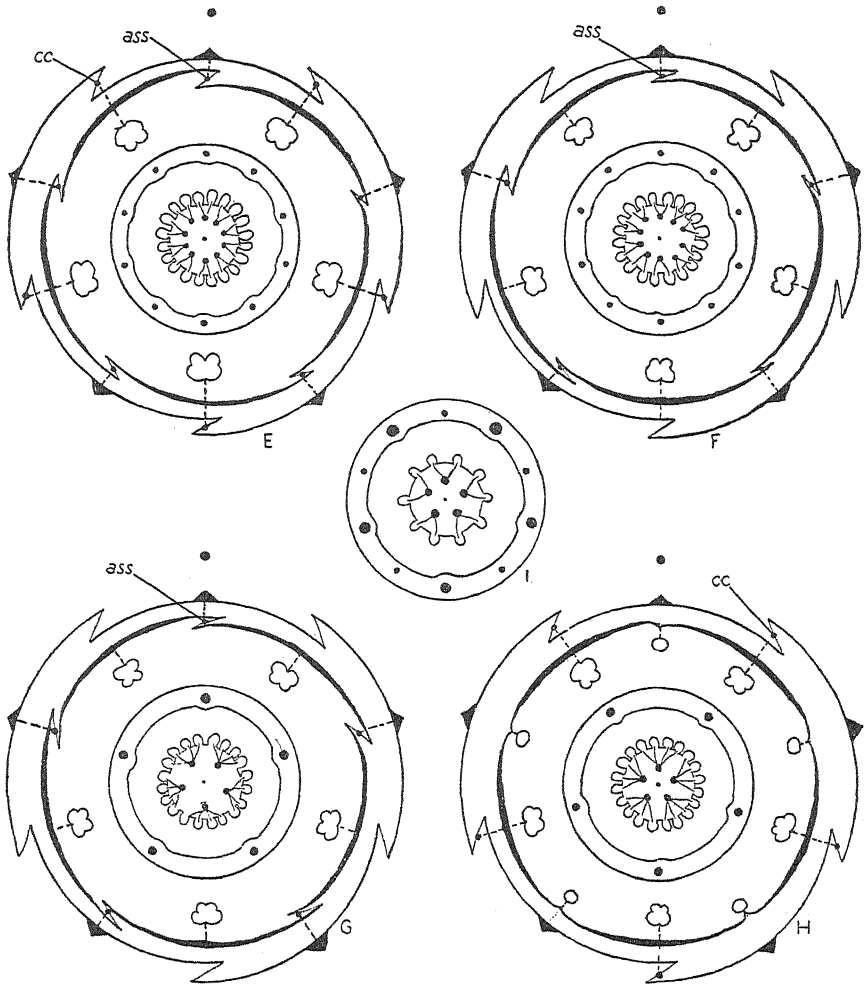
fertile carpel bundles. Van Tieghem originally investigated this feature in *Anagallis* and some of the Theophrasteae (4, p. 329) and found that these bundles have the phloem towards the inside, bordering on the central parenchyma, and the xylem on the outside. From these facts he appears to have assumed that this arrangement was general throughout the Primulaceae and to have based his interpretation of the ovary on this assumption. But in fact this is not the case. Among the genera examined an outer position for the xylem was observed in *Samolus*, *Anagallis*, *Lysimachia* and *Androsace* and various species of *Primula* belonging to the section *auricula*. On the other hand, in many other species of *Primula* (*acaulis*, *alpina*, *Cockburniana*, *darialica*, *denticulata*, *elatior farinosa*, *floribunda*, *frondosa*, Garden Polyanthus (Fig. 73), *Menziesiana*, *obconica*, *pubescens*, *saxatilis*, *sinensis*, *veris*, *verticillata*, in *Soldanella*, *Cyclamen* and *Dodecatheon*, these bundles show the arrangement with central xylem typical at a corresponding stage of the issuing bundles for the outer whorls in all these types. Now it is obvious that the ovuliferous column is of the same nature in all Primulaceae, hence it is clear that the position of the xylem in the fertile bundles cannot be taken as a guide to the morphological nature of the units composing the column. Whatever the significance of this variation may be,<sup>1</sup> it leaves the argument in support of the interpretation of the column as composed of whole fertile carpels unaffected.

*Fruit.* After what has been stated in the preceding pages regarding the construction of the ovary, little needs to be added in regard to the fruit. In the case of the capsule, thickening and cuticularization of the sectors of the wall between the bundles lead to the carpel midribs becoming lines of weakness, hence splitting takes place down these lines. If only five sterile carpels are present, dehiscence occurs only on one set of radii, that of the sepals or the petals according to the disposition of the carpels. *The five apical teeth will thus be strictly antescapalous or antepetalous as the case may be*, each tooth being composed of the conjoined halves of two adjacent carpels ( $\frac{1}{2} \times \frac{1}{2}$ ). If the typical full number (ten) of sterile carpels is present, dehiscence will lead to the appearance of five or ten teeth according as five of the midribs may be but weakly developed or even die out, or as all are equally developed and undergo splitting. In the case where all ten carpels develop but only five lines of dehiscence occur, each of the resulting five teeth corresponds to  $\frac{1}{2} \times \frac{1}{2}$  carpels. Where dehiscence takes place along all ten midribs each of the ten teeth consists

<sup>1</sup> There is some evidence pointing to the conclusion that the variation in position of the xylem in the fertile carpel bundles is associated with variation in the point of origin of the strands for the ovules. In some types these strands arise from the outer face of the bundles and run directly outwards. In the cases in which this was observed the xylem was situated towards the outside of the bundles, the bast towards the inside. In other types the ovule strands took their rise from a point much nearer the central parenchyma. In these forms the xylem was either central or on the inner side of the bundle.



(in the same way as when there are only five carpels) of the conjoined halves of two neighbouring carpels ( $\frac{1}{2}\frac{1}{2}$ ). But the teeth will now be disposed



E-I. Primulaceae. E. *Primula* type with ten outer and ten inner carpels. Calyx with commissural bundles. F. Garden Polyanthus similar to type E but without commissural bundles. G. *Primula floribunda*, Wall. Calyx without commissural bundles. Gynoecium with five carpels in each whorl, outer whorl antepetalous. H. *Samolus repens*, Pers. Calyx with commissural bundles; staminodes present; gynoecium with five carpels in each whorl, outer whorl antepetalous. I. *Anagallis arvensis*, L. Gynoecium of ten outer carpels (five antepetalous weak, five antepetalous strong) and five inner carpels. c.c., calyx commissural bundles; a.s.s., antepetalous stamen bundles.

on the ten intermediate radii, a position impossible to reconcile with the old orthodox view. In all these respects we have a precise parallel to the relations existing in the Caryophyllaceae (See above, p. 256).

the 2-whorled androecium. The two associated conditions are regarded as the response to the state of congestion arising from rapid development under limited space conditions.

6. When a gynophore, though not entirely absent, is but slightly developed the carpel midribs show a corresponding intermediate position, standing not exactly in line with the perianth members but on an intermediate set of radii. Hence, the loculi also are neither quite antesealous nor quite antepetalous, as in *Lychnis Flos-cuculi*, L.

7. In *Cerastium*, which has no gynophore, the loculi, nevertheless, stand in line with the sepals, the requisite gain in time and (vertical) ~~space~~ being obtained here through the emergence from the central cylinder of the vascular elements for perianth and androecium, not in the form of successive strands, but as a single trunk cord on each radius. Consequently when the antesealous carpel cords are due to a rise there is no check in radial development, the cords turn out in their proper sequence and the loculi appear on these radii.

8. In the isomerous genera *Spergula* and *Sagina*, in which the conditions are the same as in *Cerastium*, in so far that a gynophore is lacking and that the stamen bundles are carried out conjoined with those for the perianth, the loculi, on the other hand, are antepetalous. This opposite result is brought about as a result of the centripetal course (after dissociation from the primary trunk cords) of the stamen bundles and the consequent setting up, though by a different method, of a condition of resistance to carpel expansion on the radii of the sepals.

9. A bulky and apparently unit mass of placental vascular tissue occupies a position, almost throughout the ovule-bearing region of many types, in line with the sterile carpel midribs and loculi, having the appearance at these levels of belonging to these carpels. Each such placental mass is, in reality, composed of two portions fused together, half being derived from the neighbouring fertile carpel on each side, as is apparent at the level of origin of these strands in the stipe and also at the apex of the placental column, where, through the pith having come to an end, the individual carpels become defined.

10. Dehiscence of the capsule into teeth takes place, not along the carpel edges, but down the line of the midribs of one or both sets of carpels, these bundles constituting lines of weakness in an ovary wall in which the ground tissue has become a continuous sheet of mechanical tissue. Hence, if all the carpels split, the resulting narrow teeth will consist of the conjoined halves of two neighbouring carpels ( $\frac{1}{2} \frac{1}{2}$ ). If only one set undergo splitting the broad teeth so formed correspond to  $\frac{1}{2} 1 \frac{1}{2}$  carpels. The position of the teeth in relation to the perianth members (i.e. whether superposed or situated on the intervening radii) varies according as a gynophore is present or absent (see 4, 5, and 6 above).

11. The vascular bundles for the perianth midribs usually arise as five (or four) simple bundles which become the sepal midribs and five (or four) trunk cords on the alternate radii which furnish the petal midribs and the commissural bundles of the calyx. These latter bundles sooner or later bifurcate, the two resulting strands becoming the marginal veins of two, now disjoined, neighbouring sepals. The development of commissural calyx bundles taking their rise direct from the central vascular cylinder may be explained on the supposition that the almost simultaneous origin of the sepal and petal midribs has resulted in the elements for the marginal bundles of the sepals being in lateral contact at their origin with those of the closely adjoining elements for the petal midribs, and in their being carried out, in consequence, conjoined with these latter bundles instead of diverging as true lateral veins from the sepal midribs. It follows that the case of the caryophyllaceous calyx affords no exception to the generalization that the number of equivalent cords which emerge from the central cylinder on equidistant radii indicates the presence of an equivalent number of members in the whorl in question.

12. Calyx commissural bundles are present both in the Alsinoideae and the Silenoideae, but in the Silenoideae in which the calyx is gamosepalous the commissural bundles remain single in the gamosepalous region, whereas in the Alsinoideae, in which the sepals are soon disjoined, the commissural bundles bifurcate as soon as they arise and diverge considerably, even coming to lie in some cases (*Tunica*) alongside the midrib on either hand.

13. *Gypsophila* presents the exceptional case of a genus in which calyx commissural bundles are developed in some species but are lacking in others.

#### Primulaceae.

14. Calyx commissural bundles are commonly but not invariably present. Though this feature is apparently almost invariably constant for the species, this is often not the case with the genus (*Primula*, *Androsace*, *Soldanella*). When present these bundles carry out conjoined with them, as in the Caryophyllaceae, the vascular elements for the petal midribs.

15. The vascular elements for the antepetalous stamen bundles are always carried out conjoined with the petal midribs. Although the antepetalous stamen members are almost always suppressed, the corresponding bundles are almost invariably present, each being carried out similarly conjoined with a sepal midrib, becoming disjoined later, and after bifurcating, furnishing the marginal vein of the adjacent free portion of the petal on either side. This arrangement is met with also in *Steironema* and *Soldanella*, in which non-vascular vestiges of the lost stamens are still traceable. But in the exceptional case of *Samolus*, which has large staminodes, each disjoined bundle splits into three, the central strand entering the staminode, and the laterals, the neighbouring petals, as in other types.

16. The hitherto generally accepted view that the ovary wall is composed of five episepalous carpels from which the marginal veins have become prematurely detached to pass up into the ovuliferous column is not supported by any direct evidence, but rests on inference from the fact that the several outer whorls are pentamerous and on an interpretation of the appearance presented by certain monstrous forms, which is probably ill-founded.

17. The above interpretation fails to explain the following facts:

(a) That when the ovary wall shows five carpel midribs these bundles stand in some species in line with the sepals, and in others in line with the petals.

(b) That in many species the ovary wall shows ten equidistant bundles, five in line with the sepals and five with the petals, or an intermediate number of bundles unequally spaced.

(c) That no fixed numerical relation is observable between the bundles in the ovary wall and those in the ovuliferous column, the latter being sometimes more, sometimes equal, and sometimes less in number than the former.

(d) That the bundles in the ovuliferous column do not show that definite relation as regards their point of origin to the bundles in the ovary wall which we expect between placental strand and midrib.

(e) That the ovules are often borne in an  $\infty$  number of rows.

(f) That when the capsule, on dehiscing, forms ten teeth, these teeth do not stand in line with the ten perianth members but on the intermediate radii.

18. On the other hand, the whole body of facts fit with the following interpretation:

(a) That the full ground-plan of the gynoecium is  $G\ 10 + 10$ , though this number is often not realized.

(b) That the outer carpels are sterile and form the ovary wall and style filament.

(c) That the ovuliferous column is formed of whole consolidated carpels which may bear numerous rows of ovules.

19. When ten carpels compose the ovary wall, one set of five midribs is usually more strongly developed than the other set, and these alone are then often, but not invariably, continued up into the style filament.

20. When the ovary wall consists of only five carpels, these carpels stand in some types in line with the sepals (species of *Primula*), in others in line with the petals (*Lysimachia*, *Samolus*, *Asterolinon*, and (occasionally) *Anagallis*). This variation in position appears to be associated in hypogynous types with the presence or absence of calyx commissural bundles, which are formed in the last-mentioned genera, but are lacking in the

species of *Primula* referred to above. In *Samolus* a further complication is introduced owing to the flower being syngonous.

21. The number of fertile carpels composing the central column may be equal in number to those forming the wall, or fewer, or more numerous.

22. The midrib bundles of the fertile carpels of some types are orientated so that the xylem strand is towards the outside (*Anagallis*, *Lysimachia*, *Samolus*, species of *Primula* belonging to the section *auricula*); or these bundles may have the xylem placed centrally or towards the inside (other sections of *Primula*, *Cyclamen*, *Dodecatheon*, *Soldanella*).

23. Abnormal ovaries in which the wall appears as five leaf-like structures probably arise, not by simple disjunction of the carpel edges, but by the median splitting of the carpels on the one or other set of radii.

24. In the ripe capsule the bundles in the fruit wall constitute lines of weakness, as in the Caryophyllaceae, hence splitting takes place down the midline of the carpels. If only five sterile carpels develop, the capsule teeth are superposed upon one or other of the perianth whorls; if the full number is present, and all undergo splitting, the resulting ten teeth stand on the intermediate radii, each tooth corresponding, not to an individual carpel, but to the conjoined halves of two neighbouring carpels ( $\frac{1}{2} \frac{1}{2}$ ).

#### Myrsinaceae.

25. All the conclusions stated above in regard to the Primulaceae, in so far as they apply, hold equally for Myrsinaceae.

The relations summarized above are illustrated in the accompanying floral Diagrams.

The figures in the present account were drawn by Miss D. F. M. Pertz, to whom I here tender my grateful thanks. I wish also to express my thanks for material to the Directors of the Cambridge Botanic Garden and of the Royal Botanic Garden, Kew, to the Keeper of the Royal Botanic Garden, Edinburgh, and to the Director of the Botanic Garden, Trinidad.

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## CORRIGENDA

On Carpel Polymorphism, iv. Ann. Bot., xlv.

- p. 107. l. 14 from bottom, *for* 'In Sterculieae certain longitudinal splitting' *read* 'In certain Sterculieae longitudinal splitting'.
- p. 108. l. 25 from the top *for* 'the single or grouped member' *read* 'the single member or group'.

# Absorption and Conduction of Water and Transpiration in *Polytrichum commune*.

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With four Figures in the Text.

## INTRODUCTION.

DURING November, 1930, in the course of an investigation of the anatomical relation between gametophyte and sporophyte in mosses, observations were made on the ascent of an eosin solution in the gametophyte and its passage into the sporophyte. In *Polytrichum formosum* results were obtained clearly indicating conduction of eosin up the central strand of the gametophyte, through the foot of the sporophyte, and finally into the central strand of the seta.

Attention was redirected to the problem of the ascent of sap in *Polytrichum* by the recent publication by Bowen (1), in which the author states that 'the main water supply of *P. commune* passes up over the external surface of the plant in the form of capillary films between the closely adherent leaf-bases and the stem', and further that 'the central thickened strand in the stem of *P. commune* takes very little part, if any, in the upward conduction of water'. In view of my own observations on *P. formosum* showing a comparatively rapid rise of eosin solution in the central strand, it was decided to repeat the earlier experiments, using, however, *P. commune*, to ascertain whether this species behaved differently in view of the fact that it occupies a different ecological habitat.

### *Rise of Eosin Solution in Cut, Leafy Stems of P. commune.*

In March, 1931, 70 leafy stems of *P. commune*, having an average height of 25 cm., were cut under water and the cut ends were allowed to dip into distilled water for approximately one hour. After this period the water was replaced with a 0.25 per cent. aqueous solution of eosin. The plants were placed near a north window, and in order to prevent wilting

were covered with a bell-jar, tilted so as to admit air at one side only. To ascertain the rate of ascent of the eosin solution, stems were removed at intervals and cut into lengths of 1 cm. The height to which the eosin solution had risen could be determined easily on examination of the cut surface. In doubtful cases a longitudinal section of the segment of the stem was made, but this was necessary only in a very small proportion of the plants. In contrast to Bowen (1) the writer found no difficulty in distinguishing the colour of the eosin from that of the cell walls of the central strand, and hence this dye proved to be very satisfactory for the purpose.

The details for the first ten stems examined are recorded in Table I.

TABLE I.

Height of Stem in cm.	Time in eosin solution in mins.	Height in cm. of eosin solution in stem above level in dish.
26.5	33	6
24.5	37	11
30.0	40	9
25.5	43	14
26.0	46	8
25.5	49	14
27.0	52	9
28.5	54	22
24.0	56	11
26.5	58	11

It will be seen from Table I that considerable variation occurs in the height to which the solution rises in a given time.

In Table II average results are given for groups of ten individuals, the time required for the investigation of this number being about 30 minutes. In the last group only three plants were available owing to wilting of the remainder.

TABLE II.

No. of stems investigated in each group.	Average time in eosin solution in hours.	Average height in cm. of eosin solution in stem above level in dish.
10	$\frac{3}{4}$	11.5
10	$1\frac{1}{4}$	14.5
10	$2\frac{3}{4}$	15.5
10	$3\frac{1}{4}$	15.8
10	$4\frac{3}{4}$	17.7
3	$8\frac{1}{2}$	24.0

It is at once clear that the rate of ascent in the first hour is proportionately greater than during any subsequent period. It is possible that the cell walls are rendered impermeable by the eosin and further rise



of the solution thus prevented. This is indicated by the wilting of a considerable number of the stems, especially after five hours, and also by the small height of ascent of the solution in these cases, which was rarely more than 6 or 7 cm. Microscopic examination of the tissues showed that the solution ascended in the central strand.

Since a solution of gentian violet was used by Bowen (1) for investigation of the internal rise of liquids in *Polytrichum* stems, the above experiment was repeated, using a 0.05 per cent. aqueous solution of this dye. Of 17 leafy stems examined, the dye had in 12 cases attained a height of over 1 cm. after 23 hours, but only in one case of over 3.5 cm. Gentian violet solution, as stated by Bowen, is not well suited for this type of experiment, yet she uses it in comparing internal and external ascent of water in *Polytrichum* stems. In this connexion it is of interest that when two similar strips of filter paper are placed with their lower ends dipping into solutions of equal concentrations of eosin and gentian violet respectively, the dye of the former rises much more rapidly than that of the latter, and that in both cases the stain lags behind the ascending water, though this lag is comparatively slight in the case of eosin. It must be borne in mind, as pointed out by Pfeffer (5), that the rate of ascent of water and dye molecules in the conducting strand may be different.

*Determination of the Transpiration Rate of Leafy Stems of P. commune when External Conduction is excluded (March, 1931).*

In view of the preliminary observations recorded in Tables I and II and the statement by Bowen (1) that the central strand in the stem of *Polytrichum* plays little part in the ascent of water, it seemed essential to extend the investigation to a consideration of transpiration in these plants. Determinations of the transpiration rate of leafy stems of *P. commune*, with all possibility of external conduction eliminated, would give some indication of the degree of internal conduction. Weight-potometers were accordingly set up, the apparatus being shown in Fig. 1. It consisted of a small glass tube (A) having a capacity of 5 c.c., fitted with a cork having two perforations, one for the moss stem and the other for a fine capillary tube (B) for the admission of air. The *Polytrichum* shoot was cut under water, all dead leaves being carefully removed and the plant placed in position with the cut stem under water. At the beginning of the experiment the water (D) filled the vessel to three-quarters of its capacity, and was covered by a layer of liquid paraffin (E) to prevent evaporation. The cork was painted with a mixture of vaseline and paraffin wax, the junction between cork and stem being thoroughly sealed to exclude the possibility of external conduction. The wire (C) enabled the potometer to be easily suspended on the balance. The whole apparatus had an approximate weight of 6 to

7 grm. at the commencement of the experiment, and readings to the fourth decimal place were possible. Table III gives the results for three shoots of *P. commune* set up in such potometers, and kept under a bell-jar along with a dish of water for the duration of the experiment. No wilting of the plants occurred. Weighings were made approximately every twelve hours for four days. The average temperature of the laboratory throughout the course of the experiment was 15° C.

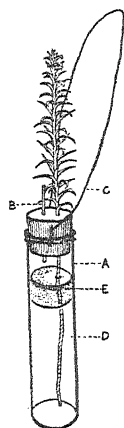


FIG. 1.  
Weight-potometer for determining the rate of transpiration of *Polytrichum commune*.

From the data given in Table III, it is clear that a steady loss of water in transpiration occurs which can only be explained by conduction through the internal tissues of the stem.

For purposes of comparison similar experiments were performed at the same time and under the same conditions with *Erica vagans*, shoots of this plant being selected because of the general external resemblance to *P. commune* in size, number, and disposition of leaves. The detailed results need not be recorded here, beyond stating that cut shoots of *E. vagans* of approximately the same size as those of the moss transpire at about one-tenth of the rate of *P. commune*. It is thus interesting to find that the moss loses water more freely than the heath.

The experiments recorded above were performed on comparatively short lengths of *Polytrichum* stem (10–13 cm.), but bearing in mind the relatively long stems of these plants, similar experiments were conducted, using shoots having a length of approximately 20 cm. but having about the same number of leaves. The results obtained were essentially the same as those given in Table III, again indicating internal conduction of water sufficient to prevent wilting under the conditions of the experiment.

TABLE III.

Time from start of experiment.		Loss in weight in grm.	Time from start of experiment.		Loss in weight in grm.	
A.			B. and C.		B.	C.
Hrs.	Mins.	A.	Hrs.	Mins.		
12	10	0.112	12	30	0.121	0.136
24	0	0.215	24	15	0.235	0.238
36	0	0.315	36	0	0.353	0.314
46	0	0.441	48	0	0.467	0.382
60	10	0.571	60	0	0.587	0.443
72	35	0.680	72	0	0.696	0.498
84	10	0.761	83	45	0.802	0.550
96	20	0.866	95	55	0.903	0.601
Height of stem		12.7 cm.			10.5 cm.	12 cm.
Number of leaves		110			85	105

Experiments with *P. juniperinum*, a moss of dry heaths, also indicated a rapid transpiration when external conduction was eliminated. The results are given in Table IV.

TABLE IV.

Time from start of experiment.		Loss in weight of potometer in grm.		
Hrs.	Mins.	I.	II.	III.
11	40	0.030	0.025	0.020
22	45	0.064	0.054	0.042
35	40	0.098	0.081	0.062
46	45	0.130	0.106	0.083
59	20	0.164	0.130	0.101
70	35	0.196	0.152	0.119
83	25	0.230	0.175	0.138
94	25	0.262	0.192	0.156
Height of stem		4 cm.	4 cm.	3.8 cm.
Number of leaves		56	48	40

Since *P. juniperinum* grows most commonly on dry open heath-land, it is to be expected that the chief supply of water for the transpiring leaves will be provided by conduction through the stem from the available water in the soil. Conditions seem unfavourable for any external conduction and absorption of water, except during periods of precipitation.

In the case of *P. commune*, however, the habitat is quite different. The plant occurs commonly in bogs where the water level not infrequently reaches the level of the lower leaves, and in such conditions external conduction, as maintained by Bowen (1), may suffice for the water requirements of the plant. It would be significant, therefore, to discover whether any internal conduction takes place when plants have the lower leaves submerged, thus providing conditions which allow of external capillary rise. This will now be considered.

*Absorption of Water by Cut Stems of P. commune with Simultaneous Conduction over the External Surface.*

For this work a special form of potometer was used (Fig. 2 a). It consisted of a long narrow glass tube (A) supporting a wider glass cylinder (B), which was closed at the lower end by a rubber bung, fitted with a capillary tube (C). This connected the two vessels, and was only sufficiently wide to allow insertion of the moss stem as shown in the figure. The narrow tube (A) containing water (E), with a layer of liquid paraffin above (F), was graduated in tenths of a cubic centimetre, and constituted the supply to the cut stem. In the upper vessel (B) water (D) covered several of the lower leaves, thus affording a supply for external conduction. The upper part of the capillary tubing and the leafless portion of stem had been securely sealed to prevent movement of water from one vessel to the other.

Air could enter the tube (A) between its upper rim and the rubber bung. The apparatus, as set up, made it possible for internal and external

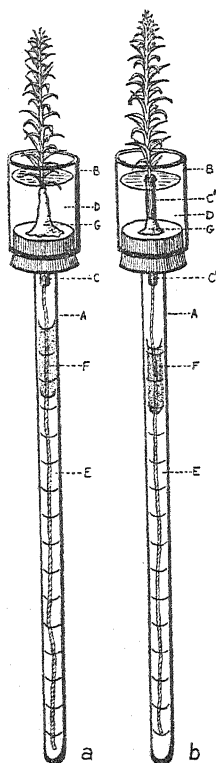


FIG. 2. (a) Potometer for measuring the rate of absorption of water by a cut, leafy stem of *Polytrichum commune* with simultaneous external conduction of water. (b) Control potometer with no external conduction.

conduction to take place at the same time. In the control experiment, the apparatus (Fig. 2 b) was constructed on the same lines, differing only in the upper part of the capillary tubing ( $c^1$ ) being extended above the surface of the water in the vessel (B), thus excluding external conduction. A set of five potometers of this type (Fig. 2 a) were set up, along with two controls (Fig. 2 b), under a large glass chamber (40 cm.  $\times$  48 cm.  $\times$  34 cm.). The average temperature of the laboratory during the period of the experiment was 15° C. The results are recorded in Table V, no allowance being made for the volume of the moss stem, hence the absorption values are slightly too high.

These results are expressed graphically in Fig. 3. It is at once evident that cut stems of *P. commune* absorb relatively large quantities of water, even when there is contemporaneous external conduction. One of the control experiments (VII) shows a considerably greater rate of absorption, while the second (VI) is not so pronounced in this respect. Subsequent experiments, however, have confirmed the results indicated by Exp. VII of Table V.

#### *Potometer Experiment in the Field (March, 1931).*

Field observations on *P. commune* indicate that the plant often exists where the water level falls below the surface of the peat, and there is little suggestion of external capillary rise. The leaves, nevertheless, remain fully expanded, except during periods of bright sunshine or dry winds. Even under these conditions plants which are protected by surrounding vegetation remain fresh, probably due to the higher atmospheric humidity among the clustered stems. In the absence of sun and wind the mutual protection afforded by the plants growing in cushions apparently raises the humidity of the atmosphere among the plants sufficiently to prevent wilting. Any individual member of a cushion will probably transpire less actively than an isolated plant.

Since the foregoing experiments were conducted with isolated shoots, it was decided to perform potometer experiments upon plants growing in the field, burying each potometer tube in a relatively large tuft of shoots,

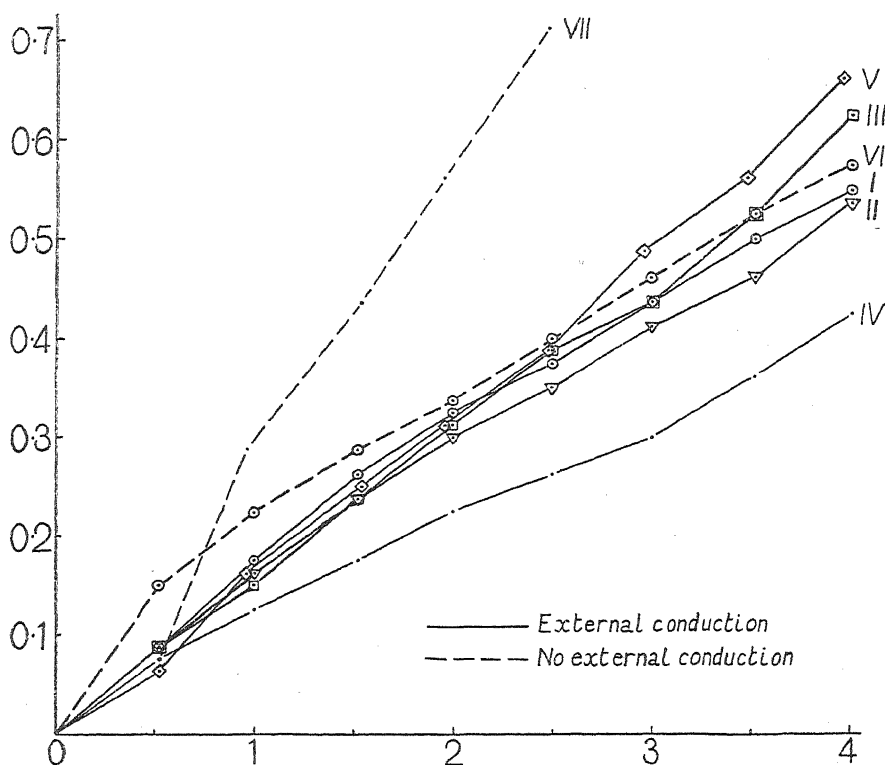


FIG. 3. Graph expressing the rate of absorption of water in c.c. by cut, leafy stems of *Polytrichum commune*, with and without external conduction of water over a period of 4 days

TABLE V.

Time from start of experiment in days.	Volume of water absorbed in tenths of c.c.						
	With external conduction.					No external conduction.	
	I.	II.	III.	IV.	V.	VI.	VII.
$\frac{1}{2}$	$\frac{7}{12}$	$\frac{7}{12}$	$\frac{7}{12}$	$\frac{3}{4}$	$\frac{5}{8}$	$1\frac{1}{2}$	$3\frac{1}{2}$
1	$1\frac{1}{2}$	$1\frac{5}{8}$	$1\frac{1}{2}$	$1\frac{1}{2}$	$1\frac{5}{8}$	$2\frac{1}{2}$	$2\frac{1}{2}$
$1\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$	$1\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$	$4\frac{3}{8}$
2	$3\frac{1}{2}$	$3\frac{1}{2}$	$3\frac{1}{2}$	$2\frac{1}{2}$	$3\frac{1}{2}$	$3\frac{1}{2}$	$5\frac{5}{8}$
$2\frac{1}{2}$	$3\frac{1}{2}$	$3\frac{1}{2}$	$3\frac{1}{2}$	$2\frac{1}{2}$	$3\frac{1}{2}$	4	$7\frac{7}{8}$
3	$4\frac{3}{8}$	$4\frac{3}{8}$	$4\frac{3}{8}$	3	$4\frac{1}{2}$	$4\frac{3}{8}$	—
$3\frac{1}{2}$	5	$4\frac{3}{8}$	$5\frac{1}{2}$	$3\frac{3}{8}$	$5\frac{5}{8}$	$5\frac{1}{4}$	—
4	$5\frac{1}{2}$	$5\frac{3}{8}$	$6\frac{1}{4}$	$4\frac{1}{2}$	$6\frac{3}{8}$	$5\frac{1}{4}$	—
Height of stem in cm.	18	19	19	19	18.5	17.5	18.5
Number of leaves	112	105	110	95	100	120	110

so that the stem used, which had a height of approximately 19 cm. and had about 110 leaves, was exposed to the same external factors as undisturbed members of the cushion. The potometer used was similar in design to that shown in Fig. 1, the glass tube, however, being much longer and

graduated to allow of volume readings. The results of a set of five such field experiments are given in Table VI.

TABLE VI.

Time from start of experiment.	Absorption of water in c.c.				
Hours.	I.	II.	III.	IV.	V.
48	0.09	0.15	0.20	0.24	0.24
93	0.24	0.24	0.39	0.61	0.70
143	0.72	0.52	0.70	0.85	1.00

*Determination of the Transpiration Rate of Plants of P. commune growing in situ.*

All the experiments so far described were performed on cut stems of *P. commune*. It was considered desirable to undertake some observations on transpiration with the plant *in situ*. This was done by enclosing the transpiring, leafy portion of the stem in an air-tight chamber, through which a stream of dry air was drawn, and then passed through weighed U-tubes containing phosphorus pentoxide, for the complete removal of water vapour evaporating from the plant. The apparatus is shown in Fig. 4. The air current was first drawn into a U-tube (D), containing phosphorus pentoxide to absorb any moisture present. It then passed through a small U-tube (A) with glass taps, also containing phosphorous pentoxide, which was weighed before and after the experiment, to show that all the water vapour had been eliminated before the air stream entered the air-tight chamber (F), enclosing the upper part of the plant. This chamber had a diameter of 3.5 cm. and a height of 6.5 cm., and both ends were closed with rubber bungs. The upper one had two holes for the entrance and exit tubes, of which the former extended almost to the bottom of the chamber, the latter just through the upper bung. The lower bung was slit along one radius to permit the introduction of the plant stem, care being taken to prevent injury to the stem by pressure. All dead leaves were removed from the shoot, and this portion was carefully covered with vaseline. The slit in the bung was also made air-tight by sealing with vaseline. On leaving the chamber the air current passed through two small U-tubes, B and C, provided with taps, and filled with phosphorus pentoxide for the absorption of water given off in transpiration by the plant. Both tubes were weighed before and after the experiment. The U-tube (E), containing calcium chloride, and connecting at H with an aspirator having a capacity of about 18 litres, prevented any possibility of a backward flow of water vapour from the aspirator into the weighed tubes, B and C. The rate of flow of air through the apparatus was regulated so that no condensation occurred on the walls of the chamber, and so that the shoot of the plant remained fresh with the leaves occupying their normal position and showing no sign

of wilting. The chamber was sheltered from sun and rain, and the temperature was recorded at intervals of half an hour. Altogether three experiments were performed: Exp. I on May 29, Exp. II on June 1, and

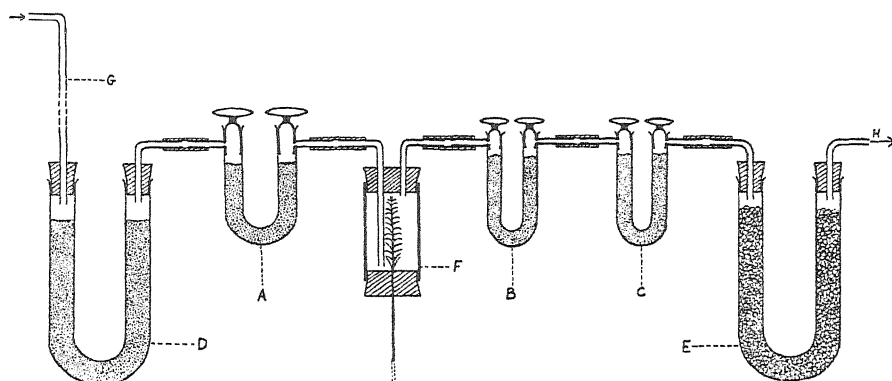


FIG. 4. Apparatus for the measurement of the transpiration rate of a shoot of *Polytrichum commune* growing *in situ*. For explanation, see text.

Exp. III from June 5 to 7. The U-tubes, A, B, and C, were first placed in a dessicator overnight before weighing to the fourth decimal place. The details are recorded in Table VII.

Details relating to the shoots used were obtained after the experiments. The stems had a length of between 25 and 30 cm. The area of the leaves was determined by drawing every tenth one to scale with a camera lucida. The sum of the areas thus outlined on paper was weighed, and also a piece of paper equivalent to a known area. From these data the total leaf area was calculated. These figures, along with those for the area of the central strand in the portion of the stem just below the chamber, are given in Table VII, and also the calculated rate of water ascent in the central strand. For the latter it is assumed that there is no change in the water content of the shoot. This probably holds good for all readings, with the exception of the first of each experiment.

The experiments show that *P. commune* in its natural habitat has a high rate of transpiration, a result which might be expected, as the lamellae on the leaves give a relatively large transpiring surface. It should be emphasized, moreover, that under the conditions of the experiment the plant was deriving its water supply entirely by internal conduction, external conduction having been excluded.

#### Discussion and Conclusion.

Haberlandt first realized the importance of the central strand for the conduction of water in certain mosses, such as *Mnium undulatum* and species of *Polytrichum*. In a paper in 1886 (3), he demonstrated this by means of the rise of eosin and other solutions in the stems. For

TABLE VII.

Exp. No.	Period.	Duration of period.	Av. temp.	Vol. of air passed through chamber. In litres.	Gain in weight of P <sub>2</sub> O <sub>5</sub> tubes in grm.			Area of central strand in I.S. Sq. mm	Calculated rate of ascent of water in central strand per hour. cm.	Total leaf area in sq. cm.	
					A.	B.	C.			Blades	Stems.
I.	1	12.10 p.m.-3.30 p.m.	17 $\frac{1}{2}$ ° C.	17 $\frac{1}{2}$	0.0006	0.1360	0.0002	0.026	157	7.0	5.9
	2	11.25 a.m.-2.25 p.m.	17° C.	13 $\frac{3}{4}$	0.0004	0.1238	0.0107	0.028	147	7.4	7.2
II.	1	2.25 p.m.-5.35 p.m.	19 $\frac{1}{2}$ ° C.	6		0.0876	0.0010		104		
	2	1.40 p.m.-4.10 p.m.	18° C.	5 $\frac{1}{8}$	0.0006	0.0658	0.0005		97		
III.	3	10.20 a.m.-12.50 p.m.	21 $\frac{3}{4}$ ° C.	5		0.0738	0.0002		109		
	4	12.50 p.m.-3.20 p.m.	25° C.	4 $\frac{1}{4}$		0.0762	0.0000		113		
	5	3.20 p.m.-5.50 p.m.	23° C.	4		0.0716	0.0019	0.027	106	6.0	5.4
	6	5.50 p.m.-8.20 p.m.	16 $\frac{3}{4}$ ° C.	3 $\frac{5}{8}$	0.0013	0.0470			70		
	7	8.20 p.m.-7.50 a.m.	—	8 $\frac{3}{8}$		0.0788	—		25		
	8	7.50 a.m.-10.20 a.m.	15° C.	5 $\frac{3}{4}$		0.0589	0.0004		87		
	9	10.20 a.m.-12.50 p.m.	16° C.	4 $\frac{1}{2}$		0.0553			82		



*P. juniperinum*, in atmospheres with relative humidities of 90 per cent. and 63 per cent., the first maintaining the plant in a fresh condition, but not the second, he found that in 10 minutes a solution of anilin blue rose 24 mm. and 42 mm. respectively. He confirmed these results with potometer experiments and obtained a transpiration loss of 0.175 gm. (an average from five experiments) per stem in 24 hours, with a temperature of  $21.2^{\circ}$ – $22.8^{\circ}$  C. and a relative humidity of 82–92 per cent. The area of the central strand in cross section, minus the area of the cell-walls, was 0.0066 cu. mm., and he calculated from this that the water ascended in the central strand at a rate of 18 mm. per minute.

Oltmanns (4), on the other hand, considered that species of *Polytrichum* and *Mnium undulatum* were chiefly dependent on atmospheric precipitation for their water supply, that their transpiration rate was low, and that the central strand was imperfectly developed as a conducting region. He quoted transpiration experiments on *M. undulatum* and *P. gracile*, set up in a cellar with a relative humidity of 94–96 per cent., and obtained values for the transpiration rates of only 0.022 gm. and 0.043 gm. per day respectively. This is not surprising in consideration of the high degree of saturation of the atmosphere.

Coesfeld (2) regarded the central strand as a region for water storage, and also reported the presence of oil drops and starch granules in the cells. The oil had been observed earlier by Oltmanns (4), and the writer is able to corroborate this.

It may be concluded from the present observations that, in *P. commune*, there is a high rate of internal conduction of water. This was first indicated by the rapid ascent of eosin solution, and is confirmed by the experimental work conducted in the laboratory and in the field. The writer agrees with Bowen (1) that *P. commune* derives some of its water supply by external capillary rise when the lower parts of the leafy plant are submerged. But these conditions do not always obtain in the field, and Bowen's conclusion that the central strand plays little part in the ascent of water is not in agreement with the experimental results here recorded. Cut stems of *P. commune* absorb water even when external water is available, and both cut stems and plants growing *in situ*, with no source of external supply, are able to maintain their shoots in an un wilted condition by internal conduction alone. How long this condition may hold remains to be determined, but the field experiment recorded above indicates a period of at least two days. It may be much longer. Certainly wilting does not necessarily occur when a supply of externally conducted water ceases, other factors being undoubtedly involved, of which the most important seems to be the humidity of the atmosphere immediately surrounding the growing plants.

Further, it is generally recognized that there is internal conduction of

water in the seta of the sporophyte; hence it would be surprising if the central strand of the gametophyte, which is more fully developed, did not serve the same purpose.

The histology of the central strand in *P. commune*, which has long been regarded as the conducting channel, has been fully described by Tansley and Chick (6), and it is therefore unnecessary to give structural details here.

#### SUMMARY.

1. The rate of ascent of an eosin solution in the central strand of *P. commune* is determined.
2. The transpiration rates for cut stems are obtained using weight potometers.
3. A potometer for investigating the rate of absorption of water by a cut stem, with simultaneous external conduction of water, is described, and results of experiments with it are given.
4. The transpiration rate of a *Polytrichum* shoot, growing *in situ*, was measured by passing dry air through a chamber enclosing the leafy portion, and absorbing the water vapour lost by the plant with phosphorus pentoxide.
5. It is concluded that the central strand plays an important part in the conduction of water in *P. commune*.

Thanks are due to Professor J. R. Matthews and Dr. C. T. Ingold for their kind advice and criticism, and also to W. R. Sherrin, A.L.S., for verifying the species of the mosses used.

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# The Absorption of Salts by Plant Tissues, considered as Ionic Interchange.

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IN an earlier paper (3) we dealt with the phenomena of the accumulation of anions and cations in the sap of plant cells.<sup>1</sup> This is probably a slow process, and may proceed mainly during the growth of the cell. The problem to be considered in the present paper is the absorption of ions over relatively short periods of time by the whole mature cell (not sap alone), or tissue of such cells, when placed in a solution of an electrolyte. There are many records of experiments on this subject, but no satisfactory explanation of the facts as a whole.

Some of the outstanding facts are as follows. The ratio of the quantity of an ion absorbed per unit volume of tissue, to the final concentration of the ion in the external solution—the *absorption ratio*—is a function of the concentration. This ratio increases with dilution, and may exceed unity for both ions of a salt. Stiles (26) suggests that the relation between the amount absorbed per unit volume of tissue and the final external concentration is expressed by the Freundlich isotherm for adsorption, and hence

<sup>1</sup> Subsequently Osterhout (23) has elaborated his idea that substances pass through the cell membrane chiefly as molecules, and that accumulation is due to the production of an acid, HA, in the cell. This production lowers the concentration of OH in the sap and KOH passes in, chiefly as molecules, tending towards an equilibrium where the product of the concentrations of K and OH in the sap equals the product for the external solution. The Cl is said to accumulate by exchange of HA for HCl, but no mechanism is suggested for forcing HCl from outside, where the product for H and Cl is about  $5 \times 10^{-9}$  into the sap, where the product is about  $5 \times 10^{-7}$ . The same difficulty arises if the cell membrane is permeable to KCl, when the ratio of the concentrations Cl, inside and out, should tend to be the inverse of that for the K ions. In the face of this difficulty of accumulating KCl against a gradient of thermodynamic potential Osterhout says there 'must be an abundant supply of energy for this purpose in the metabolism of the cell'. This is not disputed, it is the mode of application which is the real problem. Energy in itself is not enough; misapplied it could drive salts out of, instead of into, the sap. Actually there is no experimental evidence that KOH passes through chiefly as molecules, and if so, that HCl molecules cannot pass through as readily. When Valonia cells are placed in sea-water enriched with CO<sub>2</sub>, the total CO<sub>2</sub> in the sap takes about ten minutes to attain half its equilibrium value (16), whilst when NH<sub>4</sub>Cl is added to sea-water, the corresponding time is about ten days. If the carbon dioxide and ammonia pass in as molecules the cell-membrane is over a thousand times as permeable to the acid molecule as it is to the base.

concludes that absorption 'cannot be a simple process of diffusion through a membrane'.

The absorption ratio deals with quantities directly obtained from the change in composition of the external solution and the relative volumes of tissue and solution. It is a concise form of expressing experimental results, but it is not simply related to those quantities the study of which seems likely to elucidate the problem. In the first place, the amount of an ion absorbed is not necessarily the amount in the tissue, since there is the amount already in the tissue to be taken into account. Secondly, the tissue is not a homogeneous system, and hence the average concentration of an ion in the tissue is no guide to its actual concentration in any one phase of the tissue. It is in terms of concentration that an interpretation seems likely.

For the ions in the tissue which are free, the quantity  $A^c \cdot C^a$  must be the same for all phases of the tissue and external solution.  $A$  is the activity of any anion of valency  $a$ , and  $C$  that of any cation of valency  $c$ . As the total ionic concentration of any phase approaches zero the activity of an ion approaches to equality with its concentration. For our present purposes it will suffice to use concentration instead of activity. It follows that if the ratio of the concentration of a univalent ion (free to move) in one phase of the tissue to its concentration in the external solution is greater than unity, then the ratio for an ion of the opposite charge must be less than unity. There are various suggestions as to why the product of the average concentrations of two univalent ions of opposite charge in the tissue should exceed the product for the external solution.<sup>1</sup> The question of the combination of ions with substances such as the proteins we propose to consider on another occasion, it may suffice here to say that we do not think that this factor is important in the present problem.

In a recent paper Briggs and Petrie (4) showed that the explanation might be that the cell consists of more than one phase, with the necessary result that the product for the cell as a whole should exceed the product for the outside solution, except in the limit when the concentrations were the same in all phases, when the products are equal. Under certain conditions, not only is the product for the two phases together greater than that for the external solution, but the average concentration of each ion in the two phases together may exceed the concentration for the corresponding ion in the solution. For example, if the two phases of the cell are of equal volume, one containing a concentration 10 of a univalent anion and 1 of a univalent cation, the other concentrations 1 and 10, the average concentration of each ion is 5.5, and yet the cell would be in equilibrium with a solution containing each ion in concentration only 3.16.

<sup>1</sup> When the absorption ratio for each of two oppositely charged ions is greater than unity then the product of the average concentrations in the tissue must exceed that for the external solution.

It is the purpose of this paper to bring forward evidence in favour of this view of the diphasic nature of plant tissue, or rather of each of the cells of the tissue. The two phases we shall consider are the sap in the vacuole and the cytoplasm surrounding the vacuole. We shall consider the absorption of ions by the tissue on the basis of properties, the assumption of which we think is warranted by experimental facts. We do not assume that there are no other phases, or that these two have no other properties which have a determining effect on the absorption of ions. It is sufficient to explore a limited schema at the outset.

There are many analyses of plant cells or tissues as a whole, but not many for the cell-sap free from the solution in the cytoplasm. For large cells such as those of *Valonia*, *Nitella*, and *Chara* there are data (8) which show that the sap consists mainly of a solution of inorganic salts, including some or all of the ions present in the water in which the plant grows. There is evidence that the cytoplasm surrounding the sap-containing vacuole of *Valonia* and *Nitella* permits of fairly quick interchange of anions such as Cl and Br, whilst the interchange of cations is much slower, in fact negligible for the short periods such as have been used in the experiments on salt absorption with which we are now concerned.

In the alga *Valonia* a substitution of Br for Cl in the sea-water results in a considerable substitution of Br for Cl in the sap in the course of a few days (15). If this fact stood alone it might be said that the bromide rendered the cytoplasm permeable. A substitution of K for Na in the sea-water results in no significant change in the concentration of K in the sap (6) and (3). Brooks claims that the concentration of K in the sap increases, no matter whether that in the sea-water is increased or decreased. Even if these results were significant, the increased concentration of K in the sap relative to the increased concentration outside (3 to 8) is negligible compared with the figures we shall consider for the absorption by whole tissue. In any case, the increase in the concentration of Na in the sea-water does not lead to an increase in the sap, whereas Na is absorbed to a considerable extent by plant tissues as a whole. There is evidence that cations, such as Cs and Rb, which are normally absent from the sap cannot get in when the cell is mature or nearly so (9).<sup>1</sup> M. M. Brooks (5) reports a penetration of Li and Cs into the cell-sap from solutions of chlorides, the penetration being less rapid in balanced than in unbalanced solutions. The tests were spectroscopic and, as such, some of them were reported as faint even after forty-eight hours. Although precautions were taken against contamination of the sap by the external solution, it cannot be said that there is good evidence for appreciable permeability of the cytoplasm to cations.

In a previous paper (3) we suggested that the potential difference

<sup>1</sup> This is not evidence that the cytoplasm is more permeable to K, which passed into the sap at an earlier stage, than it is to Rb and Cs applied later, as the authors conclude.

between the sap and the external medium, with which the cell has reached a steady state, would give us an indication of the relative permeability to anions and cations of the membrane between sap and external solution. We do not mean a state where the cell has no activity and there is no interchange, but a state where the rate of production of carbon dioxide, &c., is relatively steady. When such a state is reached we showed that, if the sap of *Valonia* is positive to the sea-water, the indications were that the membrane as a whole is more permeable to anions than cations. In practice this P.D. cannot be determined, only that which exists when the cell is pierced by inserting a capillary into the sap, or the cytoplasm is killed at one spot, to make contact with the sap. Such treatment is likely to disturb the relatively steady state to which the cell may have previously attained. As Blinks (2) has shown, the P.D. of the sap against sea-water is very small directly after the capillary is inserted, it rises for about two days, and then decreases, the decrease still proceeding after fifteen days. Hence it is not surprising that a P.D. measured on the first day during the rise (the only data existing at the time) should be greater than the P.D. we expected from the distribution of anions between sap and sea-water. We calculated that the P.D. should be less than 2 m.v. (assuming the membrane permeable only to anions), whilst the data then existing showed the sap to be about 4 m.v. positive. Perhaps when the cell finally returns to a steady state, after more than fifteen days, the P.D. may return to a very small value.

The evidence for the ionic interchange between sap of *Nitella* and external solution is not so clear (3). Much of the work of Hoagland and Davis (12) and (13) was done with solutions of higher concentration of hydrogen ions than that to which the cells are normally exposed. Hoagland and his co-workers (14) have found that, over long periods (10–30 days) of illumination, *Nitella* cells, exposed to solutions containing KBr in a phosphate buffer, showed an increase of Br and of K in the sap. There was more of the former, the excess being balanced by a loss of Cl from the sap. But other cations, such as Na, which are absorbed by plant tissues as a whole did not pass into the sap of *Nitella*. Nor are there any reliable data, as far as we know, for the potential difference between sap and external solution with which the cell is in equilibrium. There are data for the sap against KCl solution, but here we have the complication of diffusion potentials at the interface between cytoplasm and external solution. Brooks and Gelfan (7) give data for the potential difference between an electrode in the cytoplasm and one in tap-water outside. It is not clear that the tap-water is in ionic equilibrium with the cytoplasm, and the authors produce no good reasons for their suggestion that the protoplasm forms a surface at the electrode enclosing a solution essentially like the sap. Umrath (29) has produced evidence for membrane formation at an electrode dipped in the protoplasm. Although such an electrode is positive to tap-

water outside the cell, there are too many gaps in our knowledge for us to accept the conclusion which Brooks and Gelfan draw from their experiments, that the cytoplasmic membrane is more permeable to anions than to cations.

There is evidence that the cytoplasmic membrane of some cells is permeable to cations. Osterhout (22) demonstrated the accumulation of calcium in the root-hair cells of *Dianthus*. Since such ions are normally found in the sap the membrane must be permeable to them at some stage of the development of the cell. Root-hairs, which are usually ephemeral structures, may retain cationic permeability of the cytoplasm for a longer period of their existence than do other cells.

The fact that many plant cells subsequently recover after plasmolysis in salt solutions might be an indication that both ions of the salt penetrate into the sap. It is, however, possible that the treatment has resulted in an increase of the osmotic pressure of the sap by the breaking down of more complex molecules such as starch or cane-sugar into simpler hexoses. In any case, these results necessitate the use of solutions which are relatively strong, and it is only with more dilute solutions that we shall postulate impermeability to cations. Moreover, there is a strong suggestion that the phenomenon of deplasmolysis is connected with some change in the cell brought about by the solution applied, since it does not occur if a mixture of NaCl and  $\text{CaCl}_2$  is used instead of an isotonic solution of NaCl alone.<sup>1</sup> We shall consider this question in more detail when we come to the data on salt absorption. In this connexion it is interesting to note that collodion membranes which are practically impermeable to anions such as Cl become somewhat permeable when the concentration of the salt is increased (20).

The evidence afforded by basic and acidic dyes is not easy of interpretation. In the first place, there is evidence that some molecules can pass through the cytoplasm more easily than one or other of the ions forming the molecule. Secondly, there is the size of the ion or molecule to be taken into account. A basic dye might pass into the sap, if the hydrogen-ion concentration were sufficiently low for enough of the dye to be in the unionized state, and the molecules were small enough, or sufficiently soluble in part of the cytoplasmic membrane, to pass through. Yet the fact would have no bearing on the question of permeability to the dye cations. Failure of an acid dye to penetrate might be due to the large size of both the molecules and the anions.

We now turn to a consideration of the other phase—the cytoplasm. There is much evidence that the proteins of the cytoplasm are on the

<sup>1</sup> The P.D. between the sap of *Halicystis* and external solution (Blinks (1)) is abolished by placing the cell in 0.6 m. NaCl or 0.4 m.  $\text{CaCl}_2$ . This is what would be expected if these solutions rendered the cytoplasmic membrane readily permeable to the ions of the sap and the external solution. The P.D. is still quite high if the cell is placed in a mixture of 97.5 parts of the NaCl and 2.5 parts of the  $\text{CaCl}_2$  solution; thus indicating that the permeability is not completely disturbed in this mixture.

alkaline side of their isoelectric point, that is, the protein anions predominate over the protein cations. It is the protein ions which act as the indiffusible ions.

When solutions of salts, such as KCl, are applied in two different concentrations to a cell like *Nitella* then the point of application of the stronger solution is electrically negative to that where the more dilute solution is applied (24). This happens if the cytoplasm is removed from the cell, but is more marked with the intact cell. The results are what would be expected if the K ions penetrated into the cytoplasm (not necessarily through) more quickly than did the Cl ions. The potential difference is not as great as would be expected if Cl could not penetrate into the cytoplasm. If the cytoplasm as a whole is impermeable to cations this property must reside more deeply than the external surface of the cytoplasm.

Taking all the above facts into consideration, there are some grounds for the proposition that some plant cells, over a certain range of conditions, behave as if they consisted of two distinct phases: (a) a cell-sap phase capable of exchanging anions, but not cations, with the external solution, and (b) a cytoplasmic phase containing indiffusible protein ions in excess of indiffusible cations, and capable of exchanging both diffusible anions and cations with the external solution. Superimposed on this exchange there may perhaps be a slight accumulation of both anions and cations in the sap, but the facts suggest that, even with potassium salts, this is likely to be slight, and with others probably negligible over short periods of time. It seems worth while investigating to what extent such a system can explain the phenomena of salt absorption by plant tissues as a whole when immersed in salt solutions.

#### *Absorption of electrolytes.*

When such a system as we have just postulated is placed in a salt solution it will tend towards a new equilibrium, which will be characterized by the constancy, for all phases of the cell and the external solution, of the product  $A^c.C^a$ . (see p. 302). It must be remembered that when we are considering any two phases, A refers only to those of the anions which can pass from one phase to the other, and likewise C to the cations which are diffusible. As stated before, we shall consider the concentrations as equal to the activities, for which the above statement holds.<sup>1</sup>

First, we will consider the relation between sap and outside solution and, for the sake of simplicity, we shall restrict ourselves, at the outset, to a system with all its anions univalent. Let the original concentration of the external solution of salt such as KCl be  $z$ , and its volume  $p$  times that of the sap; the concentration of Cl in the sap,  $x$ , and of the other anions (A),  $y$ . The cations in the sap will have a normality of  $x$  plus  $y$ .

<sup>1</sup> It does not matter in what form the ions move, whether separately or joined together in molecules.



Some of the anions A in the sap will pass into the external solution and their place in the sap will be taken by Cl ions from the external solution. There will be a change of the volume of the sap, if the osmotic pressure of the external solution is different from the suction pressure of the cell before it was placed in the solution. If the final ratio of the volume of the external solution to the volume of the sap is  $q$ , it can be shown that<sup>1</sup>

$$qA_0 = pz y / (pz + x + y)$$

where  $A_0$  is the external concentration of anions A which have come out of the sap. If the volume of the outside solution is sufficiently great to render the relative change of its volume, due to interchange of water with the cell, small, then  $qA_0$  can be shown to be equal to  $p(z - Cl_0)$ . This is the amount of the Cl absorbed by the sap from the solution, divided by the original volume of the sap. The expression<sup>2</sup> for the absorption ratio is

$$p(z - Cl_0) / Cl_0 = py / (pz + x).$$

It will be seen that the absorption ratio increases towards a maximum value  $py/x$  as the external concentration  $z$  approaches zero. For a given ratio of the other anions to Cl ions in the sap, it depends upon whether enough external solution has been used to bring this limiting value of the absorption ratio greater than unity. So far, nobody seems to have investigated the effect of volume of solution on the absorption ratio. As the concentration of the external solution is increased, the ratio should approach towards zero, whilst the amount of Cl ion absorbed as indicated by the decrease of the external concentration  $(z - Cl_0)$ , should approach to  $y/p$ , that is the concentration of the other anions (A) originally in the sap but now all outside, divided by the original ratio of the volumes of external

<sup>1</sup>  $v_1$  = original volume of sap

$v_2$  = final volume

$V_1$  = original volume of external solution

$V_2$  = final volume

$$\begin{aligned} v_2 A_i &= v_1 y - V_2 A_0, \quad v_2 (A_i + Cl_i) = v_1 (x + y), \\ v_2 Cl_i + V_2 Cl_0 &= V_1 z + v_1 x, \quad V_2 (A_0 + Cl_0) = V_1 z \\ v_2 A_i / V_2 A_0 &= v_2 Cl_i / V_2 Cl_0 = v_2 (A_i + Cl_i) / V_2 (A_0 + Cl_0) \\ (v_1 y - V_2 A_0) / V_2 A_0 &= v_1 (x + y) / V_1 z \\ v_1 y / V_2 A_0 &= [v_1 (x + y) + V_1 z] / V_1 z \\ q A_0 = V_2 A_0 / v_1 &= V_1 z y / [V_1 z + v_1 (x + y)] = py / (pz + x + y) \end{aligned}$$

If  $V_2 = V_1$

$$\begin{aligned} (z - Cl_0) / Cl_0 &= (V_1 z - V_2 Cl_0) / V_2 Cl_0 = V_2 A_0 / V_2 Cl_0 = v_2 A_i / v_2 Cl_i = \\ &= (V_2 A_0 + v_2 A_i) / (v_2 Cl_i + V_2 Cl_0) = v_1 y / (V_1 z + v_1 x) = y / (pz + x) \end{aligned}$$

$\therefore p(z - Cl_0) / Cl_0 = py / (pz + x).$

<sup>2</sup> We have neglected the complication of hydrolysis which will be taken up later (p. 317). If the cell were placed in pure water the sap would not retain its original composition of anions. Some would be lost to the outside solution, their place being taken by OH ions, whilst the external solution would tend to become acid. This hydrolysis of the sap would be less in the presence of salts in the external solution. As we shall see, this complication can be neglected compared with the interchange of ions other than OH in salt solutions as strong as those we shall consider.

solution and sap. This latter limit holds, no matter whether the other anions are univalent or multivalent. The absorption ratio determined experimentally varies with external concentration in the manner our theory predicts. Little would be gained by attempting a quantitative application of the equation to the existing data. There are various complications. In many cases it is clear that the values are not equilibrium values, whilst in many others there is no clear evidence on this point. Secondly, there is the leaching effect of the preliminary washing to which the tissue is often submitted. This and other disturbing factors we shall eventually consider, but an important factor which we must consider now is the effect of multivalent ions in the sap.

When the multivalent anions in the sap are taken into account the equilibrium equations become more complicated. For any particular composition of the sap, the uptake of an ion from the external solution can be obtained by graphical solution of the equations. The composition of the sap for tissues used in experiments on salt uptake is usually unknown. It is highly probable that it will be different for tissue from the same region of different individuals. The most we can do is to make calculations for a sap of composition that is possibly something like that present in tissues used in experiments on salt uptake. Although the theoretical and experimental saps may differ, the results of the calculations will not be without significance.

Czapek (10) gives figures for the ash analysis of carrot roots, the tissues of which have been much used in experiments on salt absorption. Assuming that the total ash is 10 grm. per litre of water in the tissue (an average figure) the average concentration of Cl works out at 0.014N, that of  $\text{SO}_4$  at 0.016N. About 90 per cent. of the phosphate will be in the form of  $\text{H}_2\text{PO}_4$ , if the pH of the sap is 6, and then the concentration of this ion works out at 0.017N. These will be roughly the concentrations in the sap, since, as we shall show later, the concentrations of acid radicals in the cytoplasm should be small, and the volume of the cytoplasm is probably small compared with that of the sap, in such cells. Some of the phosphorus and sulphur in the ash analysis will be derived from the proteins. We have no basis for making any allowance for this. In any case, the above figures, giving a sap 0.047N in salt concentration, will serve as a reasonable basis of calculation.

Taking the ratio of the volume of sap to external solution at 0.113 (about the figure for Stiles' experiments quoted below) calculations have been made for such a sap as the above and also for various modifications. These are recorded in Table I along with the observed values from Stiles (26). In all cases the absorption ratio is greater than unity when the initial external concentration of NaCl was 0.001N and 0.01N, and less than one when 0.1N. Allowance for the Cl ions absorbed by the cytoplasmic phase would

increase the values only little, since the concentration of these ions in the cytoplasm would be, according to our schema, smaller than the external concentration, and the volume of the cytoplasmic phase is small compared with that of the sap.

TABLE I.

*Absorption of Cl by Carrot Tissue—Hypothetical and Experimental.*

Composition of sap.	$\begin{cases} \text{Cl} \\ \text{H}_2\text{PO}_4 \\ \text{SO}_4 \end{cases}$	0.014 0.017 0.016	0.014 0.017 —	Experimental.		
Initial concentration of NaCl in solution.	<i>a.</i> Increase of Cl in tissue.	<i>b.</i> Absorption. ratio.	<i>a.</i>	<i>b.</i>	<i>a.</i>	<i>b.</i>
0.001N	0.00384	6.79	0.00377	6.57	0.00202	2.62
0.01N	0.01755	2.19	0.0126	1.47	0.0140	1.66
0.1N	0.03174	0.329	0.0164	0.168	0.07345	0.80

In comparing the calculated and observed values it should be noted that, not only may the sap of the experimental material differ from that postulated, but that our calculations are for equilibrium conditions whereas those for the experimental data may not be. Unpublished work suggests that this is probably the case. As it seems very likely that the divalent ions will take longer to reach their equilibrium distribution, calculations were made neglecting the divalent  $\text{SO}_4$  ions of the sap. There is still the same kind of difference between the calculated and experimental figures.

The figures for the 0.1N NaCl require special consideration. As pointed out earlier, the uptake of the Cl ions should approach an upper limit as the external concentration is increased. This limit expressed as increase of concentration of Cl in the tissue should equal the normality of anions other than Cl (no matter what their valency) in the tissue. For our hypothetical sap the figure is 0.033N whilst the figure from the experiments is 0.0735N. Perhaps the concentration was more than twice that assumed. If so, it appears that the tissue in the stronger solution had approached more nearly to equilibrium than that in the more dilute solutions, or else the concentration of other anions in the sap was yet higher. A more likely explanation seems to be that the cytoplasmic lining of the cells has become permeable to the cations as well as to anions. There are various reasons for this suggested explanation. In the first place there are the observations of recovery from plasmolysis in strong solutions of single salts, such as NaCl, referred to earlier. Observations of the weight of carrot tissue immersed in 0.1N NaCl in many cases show a slow loss of weight for about 48 hours (the duration of the absorption experiments we are considering) followed by a very rapid loss, whereas with more dilute solutions the tissue continues to increase in weight for several days. Stiles states

that he used maintenance of original weight as an indication of unimpaired vitality. He gives no actual figures. Our own observations show marked increase in the dilute solutions, and hence retention of original weight might be due to increase by some cells and loss by others. The cytoplasmic lining might permit of passage of cations, as well as anions, to and from the sap while retaining other substances, such as sugars, and so maintaining the turgor of the tissue.

If we assume that concentration of anions and cations is the same in the sap as in the outside solution when equilibrium is attained, we can obtain the concentration of Cl ions originally in the sap by subtracting the increase of concentration in the sap, calculated from the uptake, from the external concentration as finally measured. This gives us  $0.0917 - 0.0735$ , that is  $0.0182N$ ; a figure reasonably near to our assumed  $0.014N$ . We shall see later that this suggested explanation gives what is probably a more reasonable figure for cations in the cytoplasmic phase.

We have thought it worth while to present also the figures for uptake of Cl ions for a sap containing divalent ions increased at the expense of the univalent ions; the normality of the sap is maintained unchanged. (Table II.)

TABLE II.

*Effect of Substitution of Divalent for Univalent Anions on the Absorption of Cl—Hypothetical.*

Composition of sap.	$\begin{cases} \text{Cl} \\ \text{H}_2\text{PO}_4 \\ \text{SO}_4 \end{cases}$	0.014	0.014	0.014	0.014			
		—	0.017	0.020	0.033			
		0.033	0.016	0.013	—			
Initial concentra- tion of NaCl in solution.	<i>a.</i>	<i>b.</i>						
	Increase of Cl in tissue.	Absorp- tion ratio.	<i>a.</i>	<i>b.</i>	<i>a.</i>	<i>b.</i>	<i>a.</i>	<i>b.</i>
0.001N	0.001	1.13	0.00384	6.79	0.00416	7.85	0.00522	12.7
0.01N	0.0143	1.71	0.01755	2.19	0.01822	2.30	0.0216	2.86
0.1N	0.0321	0.333	0.03174	0.329	0.03166	0.328	0.0314	0.326

The effect of this change is to decrease the uptake of Cl ions, when the final concentration of any anion is greater in the sap than in the external solution, and to increase the absorption, if the concentration is greater in the external solution.<sup>1</sup> This point is of special interest in connexion with any investigation of the effect of concentration of hydrogen ion on the uptake of ions of a salt, such as NaCl. If the buffer is made up of phosphate with a constant concentration of each cation apart from the negligible change of H, then the variation of absorption of Na can be attributed to effect of pH on the tissue. But, in maintaining the cations

<sup>1</sup> It is of interest that in the case chosen, with the anions of the sap all divalent, the absorption ratio for 0.001N NaCl is less than that for 0.01N NaCl.

constant, the divalent  $\text{HPO}_4$  increases at the expense of the univalent  $\text{H}_2\text{PO}_4$  as the pH is increased. It makes no difference to the equilibrium distribution whether the change from univalent to divalent is made in the sap or in the external solution. Hence an increase of the pH, with such a buffer solution, would lead to an increase in the uptake of Cl if the final concentration of any anion in the sap were less than that outside, and to a decrease if the concentration of the sap were the greater, as it would tend to be with the initial outside concentration small. Apart from such a consideration as the above, one might be tempted to attribute both increase in the absorption of Na and decrease in the absorption of Cl to a direct effect of increased pH on the ionization of some weak acid or base in the tissue.

The absorption of the ions of  $\text{NH}_4\text{Cl}$  was also investigated by Stiles. The results are very different from those with NaCl, for the anion as well as for the cation. This might be considered as evidence against our suggestion that the absorption of anions was mainly an ionic interchange between the sap and the external solution. Ammonium chloride differs from sodium chloride in that the former, being the salt of a weak base, is hydrolysed in solution. Admittedly the hydrolysis in the concentrations used would be but slight—with 0.1N solution about  $7.4 \times 10^{-5}$  is hydrolysed, and about ten times as big a fraction at 0.001N. There is considerable evidence in favour of the view that molecules such as  $\text{NH}_4\text{OH}$  or  $\text{NH}_3$  penetrate quite rapidly into the sap. There is the well-known experiment to demonstrate the rapid change of the alkalinity of the sap of cells in dilute ammonia, as compared with the slow change in a similar strength of caustic soda. Recently Jacques and Osterhout (17) have investigated quantitatively the penetration of ammonia into *Valonia* cells in sea-water containing a small concentration of  $\text{NH}_4\text{Cl}$ . The ammonia molecules on reaching the sap will ionize, and give rise to hydroxyl ions, which will tend to interchange with Cl ions from outside. In as far as the pH of the sap is not very greatly changed, the extra uptake of ammonium by the sap should be balanced by an equal absorption of Cl. From the results of Stiles we see that the tissue in the 0.001N solutions reduced the concentration of the Cl of the NaCl by 0.000228, but that of the  $\text{NH}_4\text{Cl}$  by 0.000808, that is 0.00058 more. The reduction of the concentration of the  $\text{NH}_4$  was 0.00059 more than that of the Na. With the 0.01N solutions the corresponding differences were 0.00164 more Cl and 0.00211 more  $\text{NH}_4$  than Na. So the increased uptake of Cl from  $\text{NH}_4\text{Cl}$ , as compared with NaCl, can be explained on the basis of penetration of ammonia into the sap. The fact that the difference between the cations exceeds the difference between the anions is to be expected from the greater uptake of  $\text{NH}_4$  than of Na by the cytoplasm, which presumably contains Na to begin with, but relatively little  $\text{NH}_4$ .

We now turn to a consideration of the cation absorption. The cation content of carrot roots corresponding to the anion content given on an earlier page is as follows; 0.0785N potassium, 0.0682N of sodium, 0.0405N of calcium, and 0.0219N of magnesium. These figures are the average concentration for the water in the tissue. The total cations exceed the total anions by 0.16N. Some of this may be balanced by anions of organic acids in the sap. But the excess may be yet greater, since our estimate of the anions may be too great through including bound phosphorus and sulphur. Some of the calcium may be fixed in the cell-wall substances.<sup>1</sup> The remainder of the excess cations we assume to be balanced by the excess of indiffusible anions in the cytoplasmic phase of the cell. If a half, or more, of the cations are in the cytoplasmic phase the actual concentration must be quite high, since the cytoplasm occupies but a small fraction of the total volume of the cell in this tissue. If the concentration of diffusible cations is high then, unless the solution external to the cell is fairly concentrated, the concentration of anions (free to pass between cytoplasm and external solution) must be low to satisfy the fundamental equation of p. 302.<sup>2</sup>

We could make calculations for the cytoplasm similar to those made for the sap: in this case it would be for absorption of cations. Again we should find that with sufficiently dilute external solutions the calculated absorption ratio would be greater than unity, but would become less as the external concentration was increased. In the limit all the cations, of species other than the one added in the external solutions, would be expelled from the cytoplasmic phase. It is probable that long before this limit is nearly reached, the system (cytoplasm and sap as postulated) will be completely altered.

It is not worth while making these further elaborate calculations since we do not know how the different cations are distributed between the cytoplasm and sap: we have no guiding principle such as we have for deciding the distribution between cytoplasm and external solution. Between the latter two we assume interchange of cations such as K, but between sap and cytoplasm of the mature cell we assume no interchange of cations. Some information on the actual cationic composition of the cytoplasmic phase might be gained, if our schema is essentially true, from a study of the equilibrium values of the absorption of cations from solu-

<sup>1</sup> Stiles found that carrot tissue placed in M/10 NaCl lost Ca to the solution giving a concentration of 0.009N of calcium. If the solution was 8.85 times the volume of the tissue as in the other experiments this indicates a concentration of Ca in the tissue of 0.08N at least. As we shall see later we must take into account the effect of the preliminary washing to which the tissue was subjected. This washing will alter the ionic content of the tissue.

Kostytschew and Berg (18) have shown that dried carrot loses 30 per cent. of its calcium to water, and another 38 per cent. on subsequent immersion in 10 per cent. NaCl.

<sup>2</sup> In as far as the cell-wall is capable of exchanging cations with the external solution it can be treated as part of the cytoplasmic phase.

tions of sodium, potassium, calcium, and magnesium salts. Of course there would be the complication of the substances in the cell-wall. Stiles and Kidd (28) carried out some experiments on this subject but, although they talk about equilibrium values for the absorption, it is clear from their experimental records that the tissue and solutions were by no means in equilibrium. The superficial cells of the tissue in direct contact with the solution may reach equilibrium relatively quickly, but the cells inside the tissue can absorb ions only through the solution in the cytoplasm and external to it. Steward (25) found that the rate of diffusion of phosphate through tissue was very much slower than through water. There is no experimental evidence comparing the rate of divalent and univalent ions through tissue, but in specially prepared collodion membranes the divalent ions are retarded much more than the univalent (21). Hence it is possible that the rate at which pieces of tissue attain equilibrium in solutions of salts giving divalent ions may be much slower than the rate in solutions giving univalent ions.

We shall confine ourselves to a simplified case for numerical illustration. Suppose that  $6/7.85$  of the K, and  $5/6.82$  of the Na is in the cytoplasmic phase, which occupies a fraction  $v$  of the total volume, and neglect the divalent ions. The concentration of K and Na in the cytoplasmic phase is  $0.06/v$  and  $0.05/v$ , respectively. If  $v$  is no smaller than  $0.1$  the diffusible anions in the cytoplasm can be neglected, even when the external concentration is as high as  $0.1N$ .<sup>1</sup> Under such conditions the absorption of the cation Na, expressed as increase of concentration per unit volume of cell, is given by the expression  $A = 0.06 z / (0.11 + p. z)$  where  $z$  is the concentration of the salt in which the tissue is placed,  $p$  the ratio of the volume of the solution to that of the cells of the tissue, and  $A$  the absorption. For higher concentrations of the external solution, where the anionic concentration of the cytoplasmic phase is no longer negligible, the equation is a more complicated quadratic. The values for absorption of Na from  $0.001$ ,  $0.01$ , and  $0.1N$  solutions of NaCl  $8.85$  times the volume of the cells, calculated on the basis of this equation are given in Table III. The value of the absorption ratio increases with dilution from  $0.56$  to  $3.8$  and then to  $9.1$ . Comparison with the values from Stiles's experiments ( $2.98$ ,  $5.0$ , and  $5.5$ ) are not much use, since ours is frankly a hypothetical case.<sup>2</sup> But the

<sup>1</sup> The assumption of a cytoplasmic phase containing cations in a concentration as high as normal may seem absurd until it is realized that the osmotic pressure of the sap of such tissues may equal that of  $0.5$  to  $1N$   $KNO_3$ . Since the sap and cytoplasm are subjected to the same hydrostatic pressure consequent upon the turgor of the cell the osmotic (or imbibitional) pressure of the cytoplasm must equal that of the sap. If the substances such as sugars causing the high osmotic pressure of the sap are absent from the cytoplasm on account of an impenetrable layer on the inner surface of the latter (such as suggested for cations), then there must be a high concentration of cations such as K and Na in the cytoplasm in order that its osmotic pressure may be as high as that of the sap.

<sup>2</sup> Again we have neglected the part played by H and OH ions. The cytoplasm when placed in pure water would lose cations in exchange for H ions, the external solution tending to become

figures suffice to show that values of the absorption ratio ranging from a value less than unity to values greater can be explained on the basis of cationic exchange. Moreover, values greater than unity for the cation absorption can accompany values greater than unity for the anion absorption on the basis of our schema, when using not unreasonable values for the ionic composition of the sap and cytoplasmic phases.

TABLE III.

*Absorption of Na by Tissue—Hypothetical.*

Initial concentration of NaCl in solution.	Increase of Na concentration in tissue.	Absorption ratio.
0.001N	0.00506	9.1
0.01N	0.0302	3.8
0.1N	0.0603	0.56

The figures of Stiles for the 0.1N NaCl again merit special consideration. They suggest that the cytoplasmic content of cations other than Na is equivalent to 0.223N at least (expressed as a concentration per volume of cells). This would bring total cationic content of the tissue to about twice that assumed by us. This is a possibility, but there is another. To explain the Cl uptake we assumed that with the 0.1N solution the cytoplasm let through cations as well as anions. This would reduce the equivalent concentration of cations, other than Na, in the cytoplasm from 0.223 to 0.15, since the external solution and hence the sap contains 0.073N. This is much nearer our assumed figure, and, in conjunction with the evidence presented previously, is evidence in favour of our suggestion as to the change of permeability of the cytoplasm lining in contact with the stronger solution of NaCl.

This view of ionic interchange is not without experimental justification. Stiles found that the carrot tissue absorbed more cations than anions from 0.1 NaCl and, as the concentration of hydrogen ions in the external solution was hardly changed, he concluded that some cations must have passed out of the tissue. The concentration of Na in one experiment was reduced 0.017N more than that of the Cl. In the external solution he found 0.009N Ca, 0.003N K, and a trace of Mg. He says there might have been other cations to make up the 0.017, but suggests that his figures are not very exact on account of experimental error in determining the small quantities involved. Stiles made no analysis for anions other than Cl in the external solution. According to our schema there should have been

alkaline. This would happen to a smaller extent when an electrolyte was present in the external solution. This increase of H ions in the cytoplasm would depress the ionization of the weak acids present, and so tend to reduce yet further the absorption of cations such as Na. The effect of preliminary washing will be considered later.



some  $\text{H}_2\text{PO}_4$  in the external solution, and perhaps some  $\text{SO}_4$ , if it could get through the cytoplasm in time. The concentrations would perhaps have been too small to detect, for if the outside solution had been 8.85 times the volume of the cells of the tissue the final concentration of phosphate and chloride together, if all had been displaced from the sap, could not have exceeded 0.0037N ( $0.033 \div 8.85$ ).

So far we have neglected a point which we shall have to consider in connexion with the change of the conductivity of the external solution: the leaching during the preliminary washing of the tissue, and also after placing in the experimental solution.

According to the schema so far detailed, the net result of placing the tissues in the solution should be to leave the ionic concentration (in terms of normality) unchanged, except in the case where the impenetrability of the cytoplasmic membrane to cations is impaired in strong solutions. The conductivity of the external solution would then be affected only in as far as the ions coming out of the tissue had different mobilities from those absorbed by the tissue.

TABLE IV.

*Conductivity and Ionic Concentration of 0.02N  $\text{NH}_4\text{H}_2\text{PO}_4$  Solution containing Carrot Tissue.*

Time in hours.	Concentration of $\text{NH}_4$ % of initial.	Concentration of $\text{H}_2\text{PO}_4$ % of initial.	Calculated conductivity.	Observed conductivity.	Mobility of cation balancing excess of $\text{H}_2\text{PO}_4$ over $\text{NH}_4$ .
0.00	100	100	114	114	—
1.00	92.15	97.14	107.5	108	10
5.08	78.36	94.93	97.5	105	45
23.50	71.15	93.99	92.5	99	28
49.00	64.59	92.72	88.0	95.5	27

In some of the experiments of Stiles (26) the change of the concentration of the ions as well as the conductivity was determined. These measurements are of interest since, from the mobilities of the ions of the salt and the conductivity, it is possible to calculate the average mobility of the ions coming out of the tissue. The solutions of lower concentration are of more interest, since with these there seems to be less likelihood of the properties of the cells being impaired. In Table IV are the results for carrot tissue in 0.02  $\text{NH}_4\text{H}_2\text{PO}_4$ . We have added the calculated conductivity and the average mobility which the cations, replacing the excess of  $\text{NH}_4$  above  $\text{H}_2\text{PO}_4$  absorbed, must have. If this mobility had been higher than that for K (the most mobile next to H) then the indication would have been in favour of addition of anions to the external solution, to maintain the otherwise abnormally high conductivity. Actually the calculated mobility is

absurdly low : none of the cations in the tissue have such a small mobility. It does not seem likely that removal of H ions has anything to do with the state of affairs : the concentration of H ions for such a solution would have been only about  $2 \times 10^{-4}$  at the outset. Perhaps some of the phosphate was removed by calcium coming out of the tissue ; that is, removed as far as conductivity determinations are concerned, but estimated in chemical analysis. Whatever the explanation, the calculations reveal the fact that it is difficult to argue from conductivity determinations to salt absorption.<sup>1</sup>

Some of the results of Stiles and Kidd (27) on change of conductivity appear, at first sight, to be difficult to reconcile with our schema. For example, the conductivity of 0.002N KCl was reduced by carrot tissue from 658 to 436 units. This might be accounted for if most of the K was replaced by Na, the mobility of which is 0.67 that of K, and if most of the Cl was replaced by some slow-moving organic acid anion. The mobility of  $\text{HCO}_3$  is only 0.61 that of Cl, but  $\text{SO}_4$  and  $\text{H}_2\text{PO}_4$  have too high mobilities for this purpose. The difficulty is yet greater with NaCl, for the conductivity of 0.002N NaCl was reduced from 548 to 208 units. The substitution of K, Ca, or Mg for Na would increase the conductivity. It would have been interesting to have had the data for the concentration of Cl ions. In the experiments considered earlier a more dilute solution of NaCl (0.001N) lost only 23 per cent. of its Cl ions to carrot tissue in the same time, and unless there are disturbing factors, such as we met with in the ammonium phosphate, the conductivity could hardly have been reduced more than 23 per cent., possibly less.

This brings us to a consideration of the effect of the preliminary washing to which the tissue was subjected. One of the disadvantages of using cut tissue is the complication of the cut surface cells. The washing is an attempt to remove the solutes from these cut surface cells. During this washing there is a possible leaching of the intact cells which must be taken into account. There are no data which enable us to separate the effect on the cut cells from that on the intact cells. The fact that tissue already washed causes a marked increase in the conductivity of distilled water suggests that washing has been incomplete or that leaching, is still going on. If this increase of conductivity is a measure of leaching, the preliminary washing must have caused a considerable loss of electrolytes from the intact cells, since it would proceed most rapidly at first, and slow down as equilibrium was reached. If this increase of conductivity is largely a measure of leaching, then the assumption of Stiles and Kidd that the tissue loses as much to salt solutions as to distilled water is not justified.

The leaching effect depends upon many factors. It depends upon the volume of the external solution relative to that of the tissue ; upon the

<sup>1</sup> We shall suggest a possible explanation later (p. 319).

duration of the washing; upon the composition of the washing water, whether pure water, or water containing electrolytes (tap-water); and finally it depends upon whether the system being washed contains electrolytes, the ionization of which depends upon the concentration of hydrogen ions, or not.

Both of the phases of our hypothetical cell should lose ions when the tissue is placed in pure water. The sap will exchange some of its anions for OH, the salts of the sap becoming hydrolysed and the water outside becoming acid. The cytoplasm will change cations for H ions, and at its expense the external solution will gain base. But the acid groups of the cytoplasm itself (its proteins, &c.), being only weakly acidic, will combine with some of the H ions taken in, and so the hydrolysis will proceed farther than if the acidic groups had been strongly acid, and hence the external solution will gain yet more base. The base from the cytoplasm and the acid from the sap will tend to neutralize each other, and hence the hydrolysis of both phases will proceed farther than if the phases were separate. The equations for systems containing other than univalent ions are complicated; it will suffice to give simple illustrations.

If the sap contains a concentration  $c$  of a univalent salt, such as KCl, before the tissue is placed in water,  $v$  times the volume of the sap of the cells, the system will come to an equilibrium with a concentration of  $x/v$  of HCl outside and a sap consisting of  $x$  of KOH and  $(c-x)$  of KCl, where  $x$  is given by the expression

$$x^3 - c \cdot k_w \cdot v^2 + k \cdot v^2 \cdot x = 0,$$

$k_w$  is the dissociation constant for water. When  $x$  is small compared with  $c$  we have

$$x/v = \sqrt[3]{k_w \cdot c/v}.$$

With  $c$  as small as 0.04N, and  $v$  about 10,  $x/v$  is very small—about  $3 \times 10^{-6}$ . This would be a negligible increase of the external conductivity. But it should be noted that more Cl would come out if there was the OH from the cytoplasm to neutralize the HCl.

If the cytoplasmic phase consisted of a mixture of a weak acid (concentration  $P$  and dissociation constant  $k_a$ ) and its potassium salt (concentration  $c$ ) the expression for  $x/v$ , as long as  $x$  remains only a small fraction of  $c$  or  $P$ , is

$$x/v = c \cdot \sqrt{k_w / k_a \cdot P}.$$

The concentration of KOH outside is  $x/v$ , that of the protein ions decreases by  $x$ , whilst that of the protein molecules increases by the same quantity.

If the concentration of protein salt per unit volume of the tissues were 0.1 (there is 0.16 excess base in the carrot) and the cytoplasmic phase occupied but one-tenth of the whole tissue, then for  $k_a = 10^{-8}$  we should have the following values for  $x/v$ ;  $0.31 \times 10^{-3}$  when  $P$  is ten times  $c$ ,  $10^{-3}$  when  $P$  equals  $c$ , and  $3.1 \times 10^{-3}$  when  $c$  is ten times  $P$ . Part of the base

will be neutralized by acid coming out of the sap, more will be converted into salt by the continued production of carbon dioxide by the respiration of the tissue. In the experiments of Stiles and Kidd the carrot tissue after preliminary washing caused an increase in the conductivity of distilled water (about ten times the volume of the tissue) equivalent to 0.0002N KCl in one case, and three times as much in another. The preliminary washing might well have leached out a good deal more.

The continued effect of the leaching of the cytoplasm and accumulation of the anions of carbonic acid of respiration, will result in a replacement of some of the anions of the sap by the anions of the carbonic acid, in addition to the leaching effect on the sap.

As the volume of the water in which the tissue is placed is made greater and greater, the degree of hydrolysis will approach 100 per cent. Washing in running water or repeated changes of water is equivalent to using a very large volume of water.

Stiles washed the pieces of tissue for one or two days in running tap-water, and then in several changes of distilled water. The presence of electrolytes in the washing water complicates matters. If the washing water were of precisely the same composition as the solution with which the cells were in equilibrium before the tissue was cut, then washing should have no effect on the electrolyte equilibrium, provided the bulk of the washing water was so large that the electrolytes from the cut cells caused a negligible change in the washing water. Such a treatment would be ideal for removing the contents of the cut cells. If, however, the washing water was more dilute, there would be a certain amount of hydrolysis. If the salts in the washing water were different from those with which the tissue was previously in equilibrium there would be an interchange of other ions as well as of H and OH. The subsequent washing with distilled water, as practised by Stiles, would bring the tissue nearer to the state it would have reached if pure water had been used throughout.

If the washing finally results in a hydrolysis of the cytoplasm and sap, it is to be expected that the tissue will take up both cations and anions—probably more of the former—from a salt solution, the tissue giving out H ions from the cytoplasm, and OH and  $\text{HCO}_3$  from the sap in exchange. This will be extra to the interchange considered previously. The H, OH, and  $\text{HCO}_3$  ions coming out will not stay as such, and so increase the external conductivity. The H and OH will tend to combine to give water molecules, and the H and  $\text{HCO}_3$  to give carbon dioxide, which will pass out into the atmosphere.

If the washed tissue is placed in a solution of a salt such as ammonium phosphate then some of the H ions coming out of the tissue will combine with some of the  $\text{H}_2\text{PO}_4$  ions to give  $\text{H}_3\text{PO}_4$  molecules. Hence there will be a greater reduction of the conductivity of the external solution than

would be expected from calculations based upon the assumption that the ionization of the phosphoric acid remained unchanged (cf. p. 316).

Moreover, the ions which replace the ions taken up from the salt solution may be different from those expected from an analysis of the fresh tissue. Washing in water containing much Ca and little K would tend to cause a replacement of some of the K in the cytoplasm by Ca. On subsequent immersion in a solution of NaCl there may be more Ca than K lost by the tissue, although analysis of the tissue might lead us to expect more of the potassium. As we noted earlier, Stiles found a greater loss of Ca than of K from carrot tissue into 0.1N NaCl. Hence calculations of the calcium content of tissues from data of exosmosis of calcium from the tissue after washing would not necessarily agree with estimates from analysis of fresh tissue.

If the tissue is only partly leached during the washing, then in dilute salt solutions the effect of further leaching may exceed the absorption of ions by the tissue, with a consequent increase in the conductivity of the external salt solution, but clearly not to the extent with distilled water. Such was observed by Stiles and Kidd; for example, while the conductivity of distilled water was increased by 196 units, that of 0.0002N KCl was increased by only 137.

The important point is that the leaching which results from washing to remove electrolytes from cut cells may be expected to bring the tissue into such a condition that there is caused a decrease in the total concentration of ions of the external solution, and hence a greater decrease in its conductivity than could be accounted for by simple ionic interchange. It still is ionic interchange, but with the addition that some of the ions coming out of the tissue ( $H$ ,  $OH$ , and  $HCO_3$ ) are removed by combination.

Leaching will also take place in the presence of salts in the external solution, but to a smaller extent the higher the concentration of the salts. Loeb (19) investigated the equilibrium between the chloride of a weak base, gelatin, inside a collodion bag and various concentrations of  $NaNO_3$ , from zero to M/32, outside. In this case the gelatin chloride takes up  $OH$  ions from the solution outside the collodion bag, leaving the external solution more acid. With no nitrate present the outside was as acid as pH 3.05, with M/1024 only 3.11, and with M/32 yet less acid, 3.24.

There are other experimental data which can be explained on the basis of ionic interchange, although they do not necessarily imply a cell consisting of two phases. Stiles and Kidd found that the conductivity of N/50  $K_2SO_4$  was reduced much less than that of N/50 KCl or  $KNO_3$ . This is what would be expected if  $SO_4$  ions could not penetrate into the sap as rapidly as the chloride and nitrate ions. The sulphate solution would tend to have a greater negative charge relative to the cell than had chloride or nitrate solution, and hence the cation (K) would be held back

more in the sulphate solution. The mobility of the sulphate ion in solutions of the above concentration is not much different from that of  $\text{Cl}$  or  $\text{NO}_3$ , but it is the mobility through the cytoplasmic lining that is the determining factor. Elsewhere we have considered the evidence for the greater permeability of some cytoplasmic and other membranes for univalent as compared with divalent anions.

The same authors found that the conductivity of N/50 solutions of potassium salts was reduced more than that of salts of sodium, and of salts of sodium more than that of salts of lithium. This held, not only for absolute reduction of conductivity, but also for relative reduction. This is the type of result we should expect on the basis of cationic interchange, the mobility of the ions being in the order  $\text{K} > \text{Na} > \text{Li}$ . If no cations came out of the tissue, the percentage decrease should be the same for all if equal amounts of the salts were absorbed, but if cations came out in exchange the decrease should be greatest (or increase least) for the salt with the cation of greatest mobility. This is based upon the assumption that there is equal uptake of  $\text{K}$ ,  $\text{Na}$ , or  $\text{Li}$ . There is the added complication that the tissue already contained  $\text{K}$  and  $\text{Na}$ , and would take up less of these than of lithium ions. If our picture of ionic interchange is substantially true it is clear that it is difficult to argue (as do Stiles and Kidd) from changes of conductivity of different salts to rate or amount of absorption of the ions of these salts.

The fact that the conductivity of N/50  $\text{CaCl}_2$  is reduced less than that of N/50  $\text{NaCl}$  seems at first to be contrary to expectations. The mobility of  $\text{Ca}$  ions is greater than that of  $\text{Na}$  ions. Moreover, it can be shown that, if the cytoplasm behaves as a mixture of a weak acid and its salts, the concentration of the  $\text{H}$  ion in the cytoplasm should be reduced more by the  $\text{CaCl}_2$  than by the  $\text{NaCl}$ . Hence there should be a greater cationic absorption from  $\text{CaCl}_2$  than from  $\text{NaCl}$ . But at the same time the concentration of hydrogen ions in the outside solution should be increased more in the case of the  $\text{CaCl}_2$ . Since the mobility of  $\text{H}$  far exceeds that of any other cation the net result may well be a smaller decrease of the conductivity of the salt of the bivalent metal than of the univalent. For the converse case of a mixture of weak base and its salt (gelatin chloride)<sup>1</sup> Loeb (19) has demonstrated experimentally that  $\text{OH}$  concentration is reduced more by the salt of a dibasic acid such as  $\text{H}_2\text{SO}_4$  than by the salt of a monobasic such as  $\text{HNO}_3$ . At the same time more  $\text{OH}$  ions passed out into the sulphate than into the nitrate of the same normality. The effect should be yet more marked with trivalent ions of the opposite charge to the non-diffusible ions in the cytoplasm.

Actually with salts of aluminium there is a much more marked increase

<sup>1</sup> The gelatine chloride was enclosed in a collodion bag, impermeable to gelatin, and placed in the salt solution.

of conductivity than with salts of calcium. In this case Stiles and Kidd suggest that the increase is due largely to a replacement of Al ions by H ions, but they suggest no reason why it should be so, nor why it should be more marked with Al than with Ca or Na.

Thus we may conclude that there is much evidence in support of the view that uptake of salts by mature plant cells is largely a question of ionic interchange; cationic interchange with the cytoplasm and anionic with the sap.

The author is indebted to Dr. F. F. Blackman and Dr. E. J. Maskell for reading the manuscript of this paper.

#### SUMMARY.

It is suggested that salt absorption by mature plant cells from weak solutions consists essentially of an exchange of anions between solution and cell-sap and of cations between solution and cytoplasm (and perhaps wall).

Various experimental data are considered from this point of view. In strong solutions of single salts both anions and cations appear to pass through into the cell-sap. In very weak solutions the leaching effect on the cytoplasm and sap becomes important.

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# Seed Dispersal from Hygroscopic Mesembryanthemum Fruits ; *Bergeranthus scapigerus*, Schw., and *Dorotheanthus bellidiformis*, N.E.Br., with a Note on *Carpanthea pomeridiana*, N.E.Br.

BY

S. LOCKYER.

With Plate X and twenty-four Figures in the Text.

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## I. INTRODUCTION.

IN a former paper (Garside and Lockyer (3)) the fruit of *Carpanthea pomeridiana* was described with particular reference to its opening mechanism and the structure of its hygroscopic keels. An account was also given of the mode of dispersal of the seeds by falling drops of rain.

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The fruits of *Mesembryanthemum* are so diverse that their structural features are used by N. E. Brown (2) as a basis for the division of the old genus into a number of new genera. In the present paper types are described which differ from *Carpanthea* both in structure and seed-dispersal mechanism.

The fruits of *Bergeranthus scapigerus*, Schw. (*M. scapigerum*, Haw.) and *Dorotheanthus bellidiformis*, N.E.Br. (*M. criniflorum*, Linn. f.), form the subject of this paper. In these the loculi are roofed by wings, which are termed 'superlocular wings' in this paper, these being equivalent to the 'cell wings' of N. E. Brown ((1), p. 152). *Bergeranthus*, in addition to superlocular wings, possesses tubercles which close the outer openings of the loculi. N. E. Brown ((2), p. 273), remarks that '... the tubercle that nearly closes the opening of the cells (and) seems as if designed to prevent the escape of the seeds'. He also remarks that the stiff cell-wings accompanying the tubercle tend still further to prevent the escape of the seeds. He continues, 'How do the latter get out of the cells? I have failed to solve the problem; the answer to this question must be worked out by some one in South Africa.' As such wings and tubercles were not present in *Carpanthea*, the following investigation was carried out with a view to determining the structure and if possible the function of these structures, and by means of experiments with artificial rain it was hoped to elucidate the hitherto unexplained manner in which the seeds are liberated from these highly complicated capsules.

## 2. *Bergeranthus scapigerus*, Schw. (*Mesembryanthemum scapigerum*, Haw.).

This species is a succulent with numerous opposite, decussate leaves and undeveloped internodes (Pl. X, Fig. 1). The inflorescence is a dichasium, the flowers having long peduncles (Pl. X, Fig. 2). Axillary flowers are frequently undeveloped, so that a terminal flower occurs, or else a pair of flowers formed by one axillary developing in addition to the terminal. The five perianth (calyx) lobes surround the multiseriate staminodes (corolla) and the numerous stamens. The upper portion of the ovary is almost flat or, later in development, raised to form a slight cone, which is surmounted by the five thread-like stigmas.

In the mature fruit the placentation is parietal, the five carpels splitting to form five valves on the inside of each of which are two divergent hygroscopic keels. Five large tubercles alternate with the five valves, each tubercle being situated on the rim of the capsule and occluding completely the marginal opening of a loculus (Pl. X, Fig. 5).

(i) *Morphology of the fruit.*

The ripe 5-valved capsule is shown in the closed condition in Pl. X, Fig. 4. The valves do not correspond with carpels, for they rest over the septa and not over the loculi. The margins of each valve turn upwards and fit tightly against the similarly turned-up edges of neighbouring valves. In this way prominent ridges, clearly seen in Pl. X, Fig. 4, are formed between the valves. Some withered remains of the 'calyx' can be seen, but the stigmas do not persist in the ripe fruit.

When the capsule is moistened, the valves move outwards and backwards through approximately  $100^{\circ}$ . The ripe seeds (Text-fig. 1, H, and Pl. X, Fig. 3) are then easily visible in the open capsule, although they are covered by transparent, stiff, but flexible superlocular wings. Two wings, the superlocular wings (Text-fig. 1, D), arise from the top of each septum (Text-fig. 1, G) and extend more or less horizontally across the loculi to meet corresponding wings coming from other septa. Each wing is roughly triangular in shape, the longest margin being attached to the septum except for a free portion about 1 mm. towards the centre of the fruit. The second margin meets its fellow above the centre of the loculus (Text-fig. 1, E), the two edges being rather firmly pressed together but nowhere united. The angle between the first and second margins is very acute, and this portion of the wing rests upon the central columella (Text-fig. 2, J). The tip is quite free, as the wing is not attached to the septum in this region. All ten free tips fit compactly together over the columella. The third and outer margin of the triangular wing is entirely free. The space between the outer margins of the wings and the ovary wall is filled by a large, conspicuous white boss or tubercle (Text-fig. 1, A), which prevents the exit of seeds in this direction. Therefore the only possible means of seed liberation is by the lifting of the wings. Seeds are then able to pass between the margins meeting over the centres of the loculi, or from beneath the free tips of the wings resting upon the central columella.

On the inner surface of each valve is a pair of bright orange-yellow-coloured keels (Text-fig. 1, B). These keels are relatively small. Each originates from the angle of a loculus just beneath the superlocular wing, and runs from thence outwards along the inner surface of the valve for about half its length. The two members of each pair therefore originate one on either side of a septum, and they are here about 1 mm. apart. As they run outwards they diverge to become approximately 3 mm. apart at their outer extremities. The upright portion of the keel is only about 1 mm. high at its widest part, but closer observation shows that hygroscopic tissue extends as a skin on the inner side of the valve (Text-fig. 1, F). This hygroscopic skin is yellowish in colour only near the base of the keel, but may be distinguished beyond this point on account of its shining texture.

On drying, the hygroscopic tissue contracts and the valves close, ready to re-expand when again moistened. After the valves have once opened, their turned-up edges do not fit together tightly to make the prominent ridges (Pl. X, Fig. 4) seen in a fruit which has never previously been opened. Instead, the edges of each valve roll back so that the tubercles are just visible between the valves. Pl. X, Fig. 2 shows two fruits. The upper fruit is one that has closed after a previous wetting, but the lower has never been opened. From the photograph it is just possible to see the difference in the way the valves fit in the two fruits.

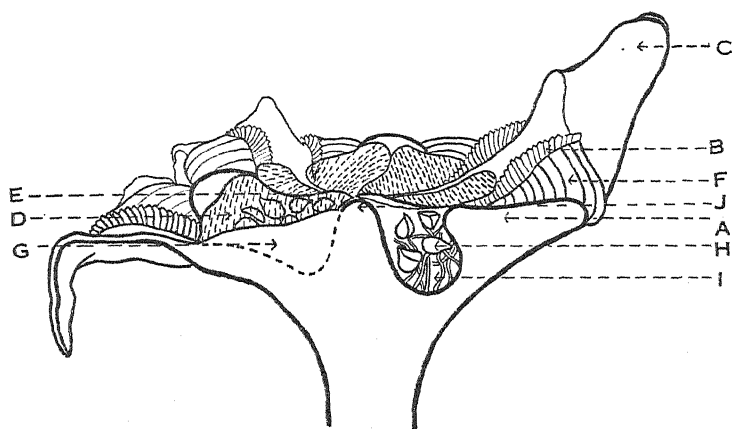
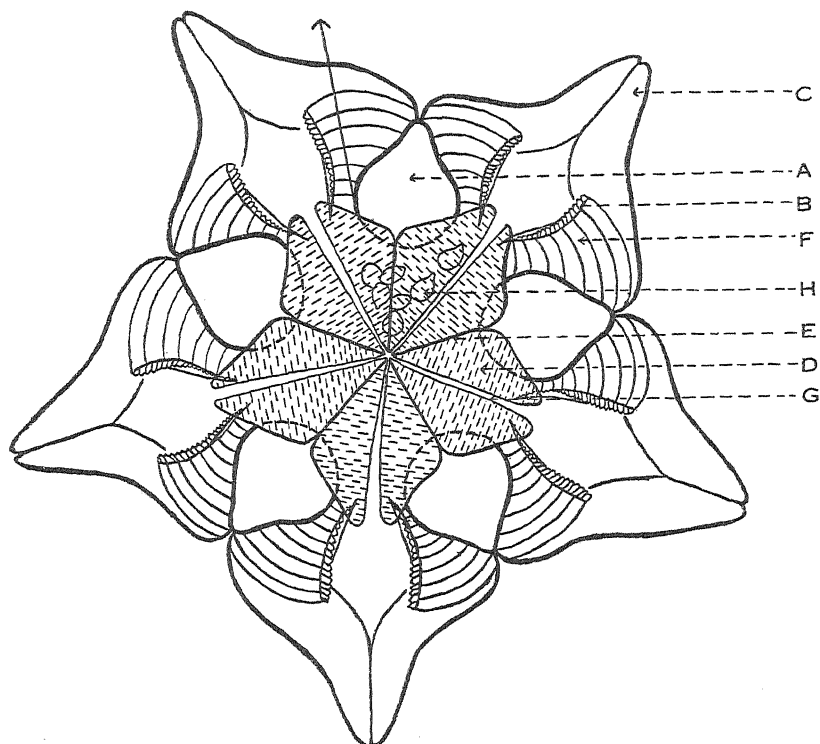
(ii) *Anatomy of the hygroscopic tissue, tubercle, and superlocular wings.*

The structure of the *hygroscopic keel* of *Bergeranthus* is similar in most respects to that of *Carpanthea* (Garside and Lockyer (3)). The primary walls of the cells, however, unlike those of the latter genus, are unthickened. The free part of the expanding keel agrees with *Carpanthea* in being only one cell in thickness, but at its widest part it is only 12–14 cells high (Text-fig. 5). The base of the keel continues over the inner surface of the valve as a single layered hygroscopic skin (Text-fig. 1, F). In surface view these cells resemble those of the keel, for they are elongated in a direction at right angles to the direction of movement (Text-fig. 3). Like the keel cells the lumen of each is almost completely blocked with mucilage, and the remains of the dead protoplast is still visible in the centre of the cell (Text-fig. 3, J). That the movement of the valve is brought about in part by this skin as well as by the expansion of the keel may be seen from Text-fig. 4. A radial section through the valve where it is covered by the hygroscopic skin, but not passing through the keel, i.e. in the direction indicated by the arrow at the top of Text-fig. 1, is shown mounted first in absolute alcohol (Text-fig. 4, i) and then in water (Text-fig. 4, ii). Expansion of the hygroscopic skin on mounting in water has caused the tip of the valve (a) to move through nearly 90°.

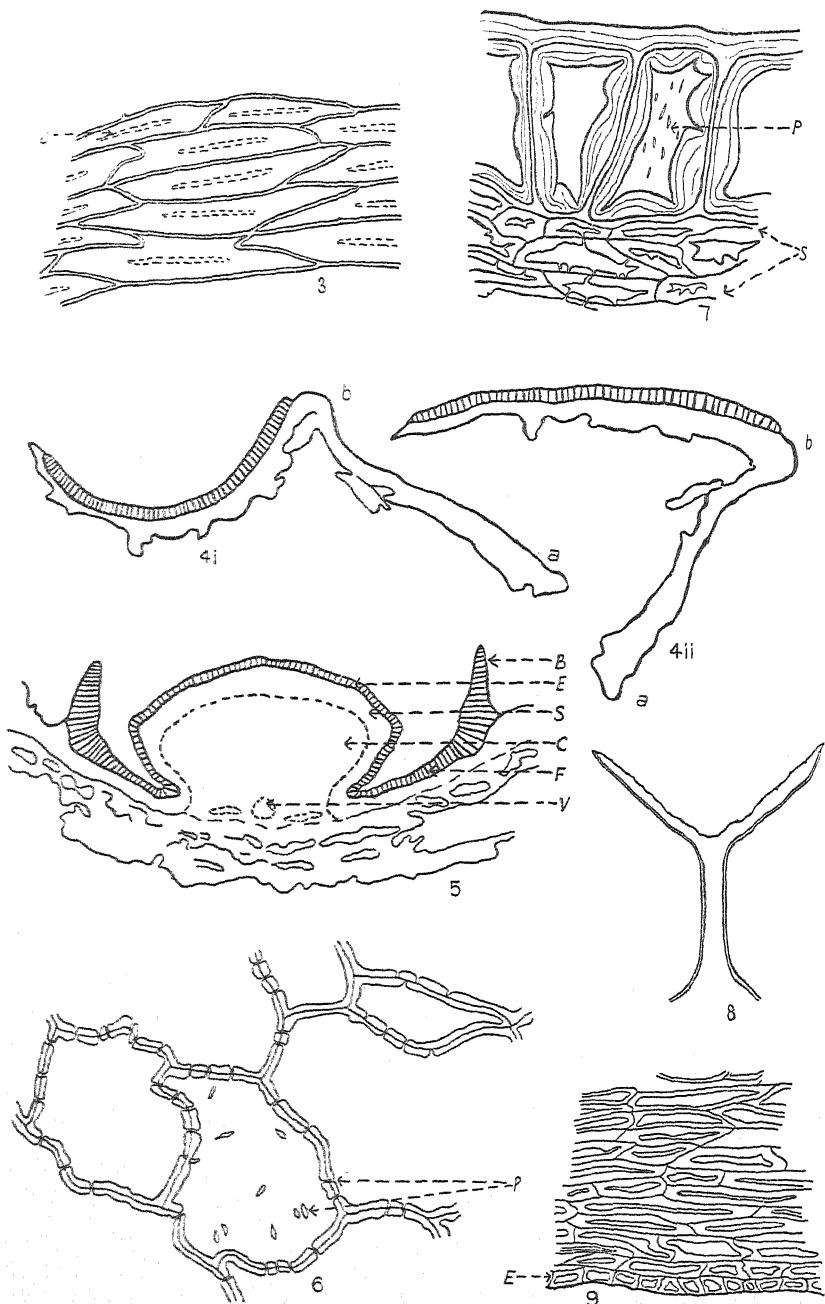
Sections show that the *tubercle* is differentiated into three regions, namely, epidermal, subepidermal, and a central region (Text-fig. 5). The cells of the central region are large and empty, and have strongly pitted walls (Text-fig. 6). About one-third of this region gives a cellulose reaction (blue with Schulze's solution), but the lower two-thirds, including the region round the vascular bundles (Text-fig. 5, v) are lignified (red with phloroglucin).

The subepidermal region (Text-fig. 5, s) is about three cells in thickness. The cellulose walls of the constituent cells (Text-fig. 7, s) are heavily pitted, and frequently so thick that the lumen of the cell is almost obliterated.

The epidermis of the tubercle is continuous with the hygroscopic skin of the valve (Text-fig. 5). The epidermal cells covering the tubercle,

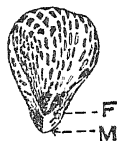


TEXT-FIGS. 1 and 2. 1. Open fruit of *Bergeranthus scapigerus* seen from above. A. Tubercle. B. Hygroscopic keel. C. Valve. D. Superocular wing. E. Free margins of wings meeting above the centre of the loculus. F. Hygroscopic skin. G. Septum. H. Seed seen through the transparent wing.  $\times 5$ . 2. Half of an open fruit of *B. scapigerus* viewed obliquely. I. Loculus. J. Columella. (Other letters as in Fig. 1.)  $\times 5$ .



TEXT-FIGS. 3-9. 3. Surface view of cells of the hygroscopic skin in expanded condition. J. Remains of dead protoplast.  $\times 250$ . 4. Vertical section through valve and hygroscopic skin. (Direction of section indicated by the arrow at the top of Fig. 1.) (i) Mounted in absolute alcohol. (ii) Mounted in water.  $\times 12.5$ . 5. Vertical transverse section through tubercle and

however, have thin primary walls and thick pitted secondary walls of stratified cellulose (Text-fig. 7). Unlike the cells of the hygroscopic skin the lumen is not completely blocked, and no dead protoplasmic remains are to be seen. If a section of the tubercle be transferred from water to



TEXT-FIG. 10.



TEXT-FIG. 11.



TEXT-FIG. 12.

TEXT-FIGS. 10-12. Seeds of *B. scapigerus*. 10. Seed viewed from above. 11. Embryo dissected from the seed. 12. Seed seen from the side. M. Position of micropyle. F. Point of attachment of the funiculus.  $\times 15$ .

absolute alcohol the epidermal and subepidermal regions contract so that their walls appear considerably wrinkled. There is no appreciable change in shape or size of the tubercle as a whole, however, owing to the rigid nature of the central region.

Finally, the structure of the superlocular wings remains to be considered. A vertical section through a septum with its attached wings is shown in Text-fig. 8. Each wing is approximately twelve cells thick (Text-fig. 9), the lower layer forming a more or less definite epidermis (Text-fig. 9, E). The upper surface on the contrary is ragged, which suggests that this face of the wing has become torn away from some other tissue during its development. The cells have thick cellulose walls and no cell contents, their shape is so irregular that the limit of each cell is difficult to determine.

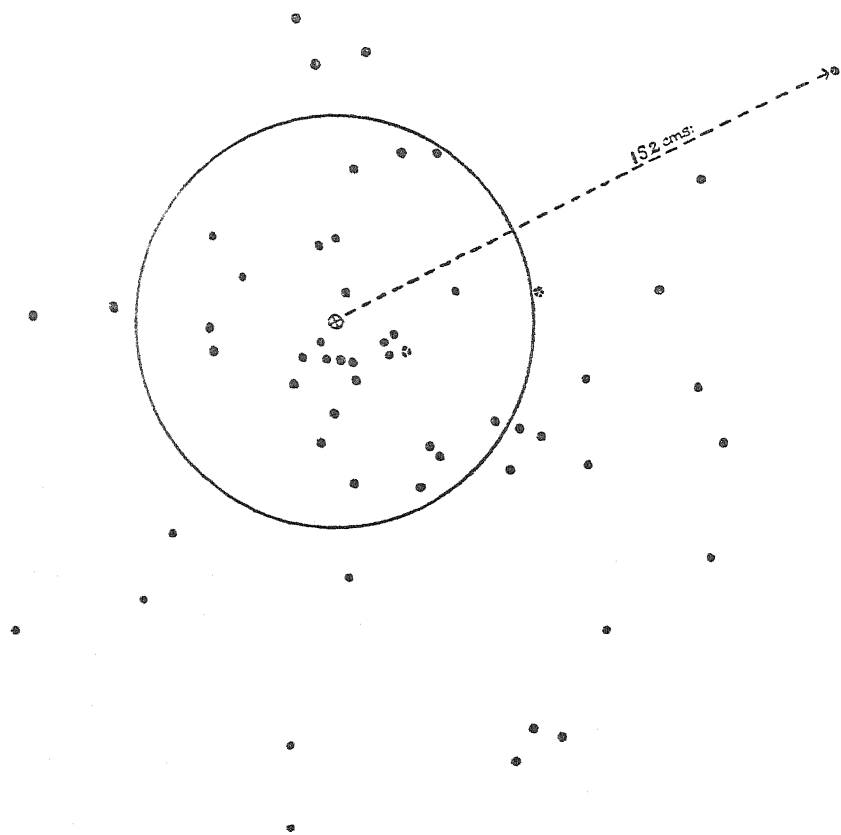
### (iii) *The Seed.*

The campylotropous seeds (Pl. X, Fig. 3) are pale yellow in colour with golden brown markings. They have two approximately D-shaped, somewhat flattened sides which are joined along their convex margins by a third side, immediately beneath which is situated the embryo with its broad cotyledons (Text-fig. 11). The oil-containing embryo is curved above a starch-containing parenchyma (interpreted as perisperm by Huber (4)). The position of the micropyle (Text-fig. 10, M) as well as the point of attachment of the funiculus (Text-fig. 10, F) can be recognized externally. There are variations both in the size and the shape of individual seeds. This is probably accounted for by the fact that they develop in closely crowded loculi, and are therefore likely to be modified by mutual pressure. In length the seeds average 1.08 mm. and 0.72 mm. in height.

adjoining hygroscopic keels. B. Hygroscopic keel. C. Central region of tubercle. E. Epidermis. F. Hygroscopic skin. S. Subepidermal region of tubercle. V. Vascular bundle.  $\times 12.5$ . 6. Vertical longitudinal section of the central cells of the tubercle. P. Pits.  $\times 250$ . 7. Longitudinal section of the epidermal and subepidermal regions of the tubercle. P. Pits in the epidermis. S. Subepidermal region.  $\times 250$ . 8. Vertical section through a septum with its attached superlocular wings.  $\times 12.5$ . 9. Transverse section, superlocular wing. E. Epidermis.  $\times 150$ .

(iv) *Experimental results.*

In order to observe the manner of seed dispersal from capsules of *Bergeranthus* an arrangement was made for providing artificial rain. A

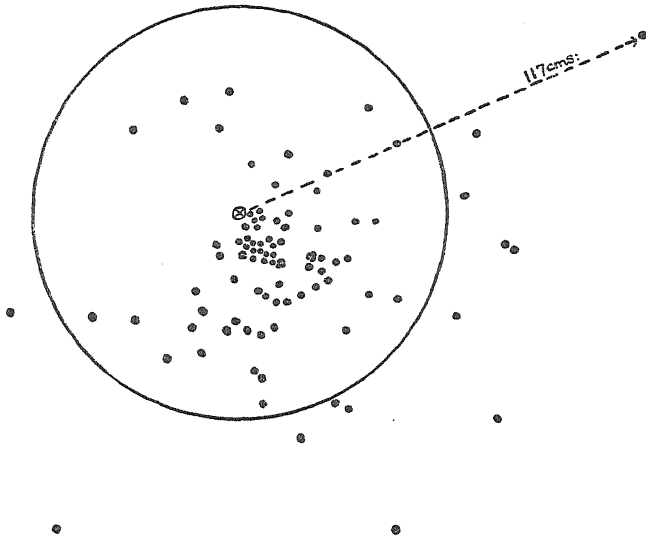


TEXT-FIG. 13. Chart of the distribution of sixty-two seeds scattered by artificial rain from an intact capsule of *B. scapigerus*. Equal numbers of seeds are within and without the circle. Scale 1 in 20.

large glass bottle, filled with water and placed on a high shelf, was connected by rubber tubing with a glass dropping tube suspended from the centre of the ceiling. The drops (radius 0.269 cm.) fell from a height of two metres, and the rate at which they fell (40 per half-minute) was regulated at the beginning of each experiment by means of a tap on the tube. The floor of the room was covered with white oil-cloth marked in 10 cm. squares, and each square was numbered. It was, therefore, possible to find all the seeds at the end of each experiment, and to make an accurate record of their position on squared paper.



*Experiment I.* An open capsule with its stalk fixed in wax was placed under the falling water drops. Immediately seeds began to be ejected by the water drops, at first rapidly and then more slowly. After ten minutes the experiment was discontinued, and an examination of the capsule showed that only eight seeds remained entangled with the long funiculi at the bases of the loculi. The sixty-two seeds which were dispersed from the capsule



TEXT-FIG. 14. Chart of the distribution of ninety-four seeds from a capsule of *B. scapigerus* from which the superlocular wings had been removed. Six times as many seeds within as without the circle. Scale 1 in 20.

fell in the positions shown in Text-fig. 13. The farthest distance that a seed was ejected was 152 cm. The diagram illustrates the very even way in which the seeds are distributed around the capsule, which is marked with a cross. Although the small seeds were closely packed together in the capsule they separated during dispersal, with two exceptions: in one case three seeds, and in the other five seeds, were found in the same drop of water (Text-fig. 13).

When the capsule is completely full of water a drop falling with force on to the flexible superlocular wings beats them downwards and ejects a fine spray of water from the flooded loculus. The movements of the wings force the seeds towards the centre of each loculus, and they subsequently escape as the water splashes through the crack between the halves of each pair of wings, or from under their free tips which rest upon the columella. That the seeds must necessarily escape towards the centre or through the crack between the two superlocular wings is obvious, because the large tubercle fills the gap between the wings and the outer ovary wall, thus completely preventing peripheral ejection.

The regular distribution of separate seeds around the capsule raises the question as to whether more than one seed can escape as the result of a single drop falling on the capsule (excluding cases where seeds actually adhere together, e.g. by funiculi, and are therefore ejected as a single particle). The following experiment was carried out to determine this.

*Experiment II.* The rate at which drops fell was slowed down, and after a known number of drops had been seen to strike the capsule the latter was covered until the seeds had been collected. The cover was then removed, and a few more drops allowed to strike the capsule. The seeds were again collected. This was repeated several times with the following result:

Capsule I.		Capsule II.	
No. of drops.	No. of seeds distributed.	No. of drops.	No. of seeds distributed.
3	1	1	0
5	5	1	2
5	2	1	2
1	1	1	1
1	1		
5	2		
1	2		

The above tables show that in three cases more than one seed was scattered as a result of a single drop falling on the capsule. In each of these cases the seeds collected were found to be well separated from each other, and must therefore have been carried away from the capsule in separate drops of water. Although a fine spray of water is seen as each drop strikes the capsule, it is impossible for several seeds to be carried away simultaneously by these numerous drops, for the slit between the halves of each pair of wings is too narrow to permit the escape of several seeds simultaneously.

*Experiment III.* In order further to test the influence of the wings on seed ejection, a capsule was opened and the wings were carefully removed with a pair of forceps, before the capsule was put under the falling water drops.

In this case all the seeds were dispersed in less than three minutes, although the water was dropping at the same rate as in Experiment I, but how ineffectively in comparison with those from an intact capsule can be seen by comparing Text-fig. 14, which represents the effect of removing the wings, with Text-fig. 13, which represents distribution from an intact capsule. In the case of the intact fruit (Text-fig. 13) equal numbers of seeds fell inside and outside a circle of 52 cm. radius. In the case of the capsule without wings, however, six times as many seeds fell within as without a circle of the same radius.

In the latter case the farthest distance a seed travelled was 117 cm.

Text-fig. 14 shows only three cases where more than one seed was carried in a single drop of water, but the repetition of the experiment with a capsule containing 161 seeds showed no less than twelve such cases.

The above experiments, therefore, prove that the wings do function for a more efficient distribution of the seeds. Without the wings only a small proportion of the seeds is splashed a considerable distance, but the majority is merely flooded out of the loculi and consequently falls nearer the capsule. In this way the capsule is rapidly emptied, but ejection to a distance impaired.

When superlocular wings are present the dispersal is slower, for the seeds have, as it were, to 'wait their turn' to pass through the narrow slits between the wings, or from beneath their free tips. The result is that the seeds have less opportunity of clinging together when leaving the capsule. The majority of seeds also falls farther afield, for they are carried in the spray which results from the beating of the wings on the flooded loculi. That the wings are a device for preventing all the seeds from becoming dispersed in one shower I do not believe. Experimentally a capsule has been emptied (except for eight seeds) in ten minutes when the drops were falling at the rate of forty per half-minute, so that a heavy storm of rain should accomplish the same result in much less time.

3. *Dorotheanthus bellidiformis*, N.E.Br. (*Mesembryanthemum criniflorum*, Linn. f.).

This plant is a herbaceous annual which branches from the base, and with leaves covered with small epidermal water storage cells. The flowers are solitary on peduncles which are longer than the leaves. The long-tipped calyx lobes, also covered with small water storage cells, are of uneven length, and surround the single row of staminodes (petals) (Text-fig. 15). The stamens are in two rows, with the inner row geniculate and resting upon the flat top of the ovary. In the flowering stage the stigmas are broom-like, and the ovary has five loculi with parietal placentation. Wings roof the loculi of the 5-valved fruit, but tubercles are absent.

(i) *Morphology of the fruit.*

Externally the ripe capsule differs markedly from that of *Bergeranthus*. The broad flat base of the ripe capsule rests firmly on the ground, on account of the bending of the peduncle after fertilization (Text-fig. 15). The dry withered calyx persists, and even in the ripe fruit the five large unequal calyx lobes form a prominent feature (Pl. X, Figs. 6 and 7). The stigmas also persist, they are hard and woody, and each is split longitudinally. When the capsule is moistened the valves move outwards and backwards.

They reflex until the tips of the persistent stigmas come to rest upon the surface of the ground, and so help to support the open capsule in a horizontal position (Pl. X, Fig. 7). As each valve is made up of the halves of



TEXT-FIG. 15. Whole plant of *Dorotheanthus bellidiformis* showing one open flower, and one young fruit whose peduncle has begun to bend. Natural size. (From a drawing made by Mr. Garside from dried material.)

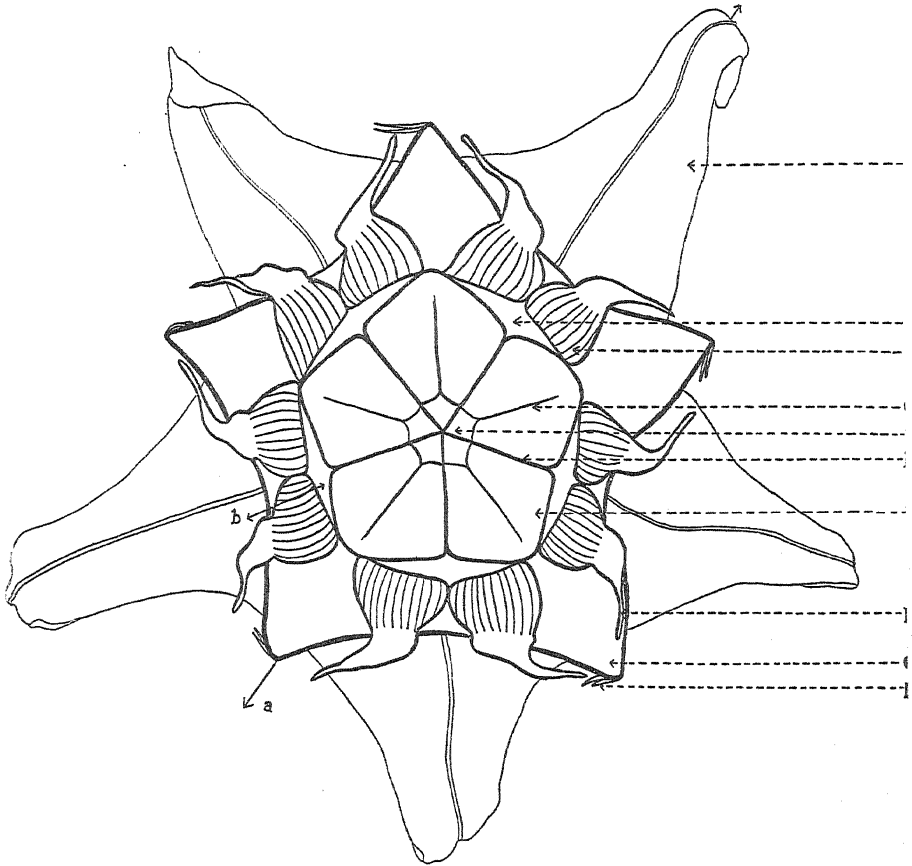
two adjacent carpels, each has attached to its tip the corresponding halves of the two stigmas.

When the fruit is open (Pl. X, Fig. 7) the hygroscopic tissue is less easily recognized than is the case in *Bergeranthus*, for the colour is inconspicuous. Further, instead of a vertical fan-shaped keel being present, the hygroscopic tissue is attached like a skin to the inner face of the valve, and so resembles the hygroscopic skin of *Bergeranthus* without the upright keel. Two triangular areas of this skin are attached to the inner surface of each valve (Text-fig. 16, F), but the apex of each triangle is prolonged into a free pointed tip (Text-fig. 16, B).

As in *Bergeranthus* superlocular wings (Text-fig. 16, J) are present, a pair of wings arising from the top of each septum (Text-fig. 16, G) and extending from thence half-way across the adjacent loculi. The central columella (Text-fig. 18, I) is large and corky, and the inner angles of the wings rest against it. Unlike *Bergeranthus* the wings extend almost to the ovary wall, for no tubercle is present. If the edges of the wings are lifted, however, a small rim can be seen to project from the ovary wall to form a narrow bracket (Text-fig. 18, A) on which the outer margins of the wings rest. This shelf occupies a similar position to the tubercle of *Bergeranthus*.

(ii) *Anatomy of the hygroscopic tissue and superlocular wings.*

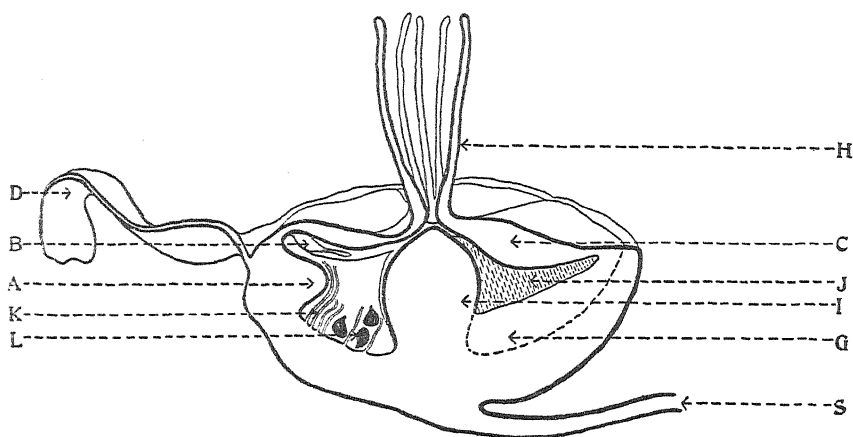
The paired superlocular wings arise from the tops of very low septa, and as they are inclined at an angle of about  $60^\circ$  to the horizontal (Text-



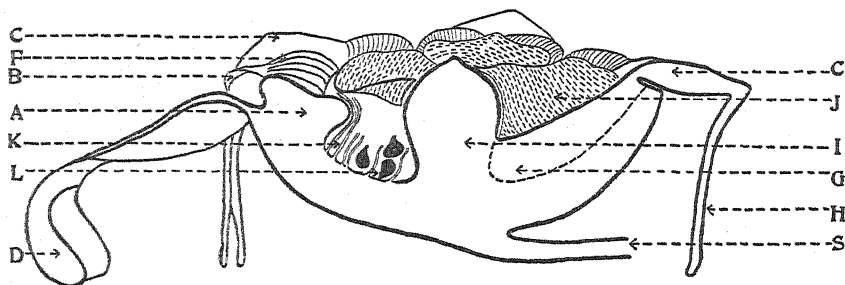
TEXT-FIG. 16. Open capsule of *D. bellidiformis* seen from above. A. Narrow shelf projecting from ovary wall. B. Free tip of hygroscopic tissue. C. Valve. D. 'Calyx'. E. Free margins of superlocular wings meeting above the centre of the loculus. F. Hygroscopic tissue. G. Septum. H. Stigma. I. Columella. J. Superlocular wing.  $\times 3.3$ .

fig. 19) the positions of the septa, in the open fruit, are marked by deep grooves. In anatomy they are similar to those of *Bergeranthus*, in that the upper surface has no well-defined epidermis above, but is covered by an epidermis below. The cell walls are of cellulose, but are very thin in comparison with those of *Bergeranthus*, consequently the wings are more flexible and less resilient. Towards the free margins of the wings the cells are so contorted and collapsed that their limits are difficult to discern. In the central region of the wings, however, six or seven rows of cells can be counted.

The *hygroscopic tissue* differs markedly from that of both *Carpantea* and *Bergeranthus* for, as already stated, there is no upright free portion forming a keel. Instead, all the tissue forms a flat skin attached to the



TEXT-FIG. 17.



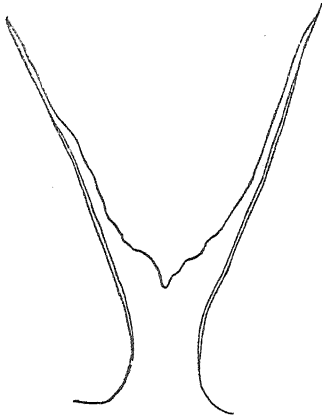
TEXT-FIG. 18.

TEXT-FIGS. 17-18. 17. Half fruit (viewed obliquely) of *D. bellidiformis* in the closed condition (cut along the line *a*, Fig. 16). K. Funiculus. L. Seed. S. Stalk. (Other letters as in Fig. 16.)  $\times 3.3$ . 18. Half fruit illustrated in Fig. 17 after it has been expanded in water. (Lettering as in Figs. 15 and 16.)  $\times 3.3$ .

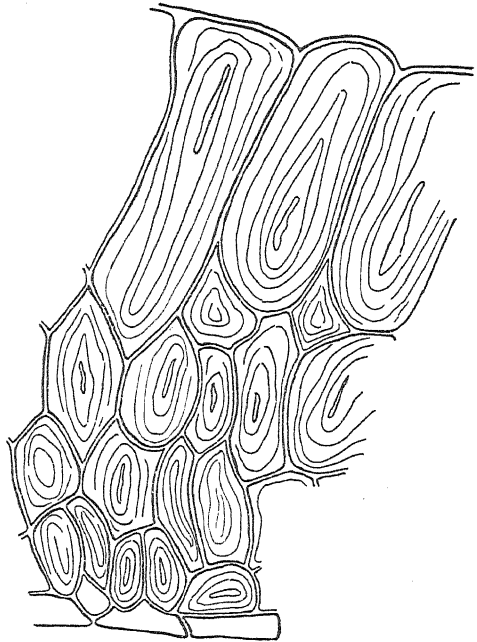
inner side of the valve (Text-fig. 16, F). It differs from the 'hygroscopic skin' of *Bergeranthus*, however, in that it is not one but four cells thick (Text-fig. 20). The primary cellulose walls are thin, and the lumen of each cell is almost completely obliterated by a thick secondary deposit of lamellated mucilage, although in most cases the remains of the protoplast can still be seen in the centre of the cell. The outer cells have palisade form, but the lower layers are rounded or pentagonal when seen in vertical section.

The hygroscopic tissue (Text-fig. 16, F) continues to the base of the narrow shelf (Text-fig. 16, A) which projects from the ovary wall. The

cells of this shelf are large and devoid of contents. They are similar to the cells of the central region of the tubercle of *Bergeranthus* (Text-fig. 6).



TEXT-FIG. 19.



TEXT-FIG. 20.

TEXT-FIGS. 19-20. 19. Vertical section through a septum of *Dorotheanthus* with its attached wings.  $\times 12.5$ . 20. Vertical section through the hygroscopic tissue of *Dorotheanthus*. (Cut in direction *b*, Fig. 16.)  $\times 250$ .

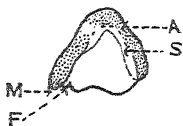
### (iii) *The seed.*

The pale grey campylotropous seeds have two D-shaped flattened sides which are slightly transparent, so that the shape of the embryo is visible through the testa (Text-fig. 21, S). These D-shaped sides are joined along their convex margins by an opaque white band which gives to the seeds their very striking appearance (Pl. X, Fig. 8). The oil-containing embryo lies immediately below this opaque band of the testa and is curved above a starch-containing parenchyma, interpreted by Huber as perisperm. These characters of the seed are probably generic rather than specific, because the seeds of *D. bellidiformis* are similar to those of *D. gramineus*, Sch., which were examined for comparison. The seeds of *D. bellidiformis* average 0.93 mm. in length and 0.7 mm. in height.

### (iv) *Experimental results.*

Seed dispersal from fruits of *Dorotheanthus* was tested in the same way as for *Bergeranthus*. Text-fig. 23 illustrates the dispersal of seeds from an

intact capsule, the capsule marked by a cross, and may be compared with Text-fig. 13 for *Bergeranthus*, for in both cases the artificial rain was continued for ten minutes. During this time 159 seeds were scattered, but many



TEXT-FIG. 21.



TEXT-FIG. 22.

TEXT-FIGS. 21-22. 21. Seed of *D. bellidiformis* seen from the side. A. Opaque white band curved above the embryo. S. Embryo visible through the transparent testa. M. Position of micropyle. F. Point of attachment of the funiculus.  $\times 15$ . 22. Embryo dissected from the seed.  $\times 15$ .

seeds still remained in the capsule at the end of the experiment. The greatest distance to which a seed was ejected was 164 cm.

The wings of a second capsule were removed before it was put under the dripping water. In this case, as also in *Bergeranthus*, the seeds were dispersed in less than three minutes, but the majority fell nearer the fruit than was the case for the intact capsule. 115 cm. was the farthest distance that a seed was ejected.

The wings of *Dorotheanthus* are less rigid than those of *Bergeranthus*. Rain falling with force into the troughs above the septa beats against the wings, and seeds are expressed in a fine spray of water from the flooded loculi. When the wings are removed the seeds are merely washed out from the capsule, instead of being sieved out, as it were, between the wings. Consequently they are less evenly distributed.

Seed dispersal in the case of both the genera described depends upon the force of the falling raindrops beating upon the wings and so splashing out the seeds. In order that the maximum advantage may be obtained from the falling rain the capsules need to be suitably orientated. This is attained in both cases, although the methods are very different in the two genera. In *Bergeranthus* the peduncle is long, upright, and very rigid (Pl. X, Fig. 1). Consequently, when the capsule opens, its upper surface, is horizontal. When the rain falls the peduncle vibrates but does not bend with the force of impact, and hence the seeds are readily splashed out.

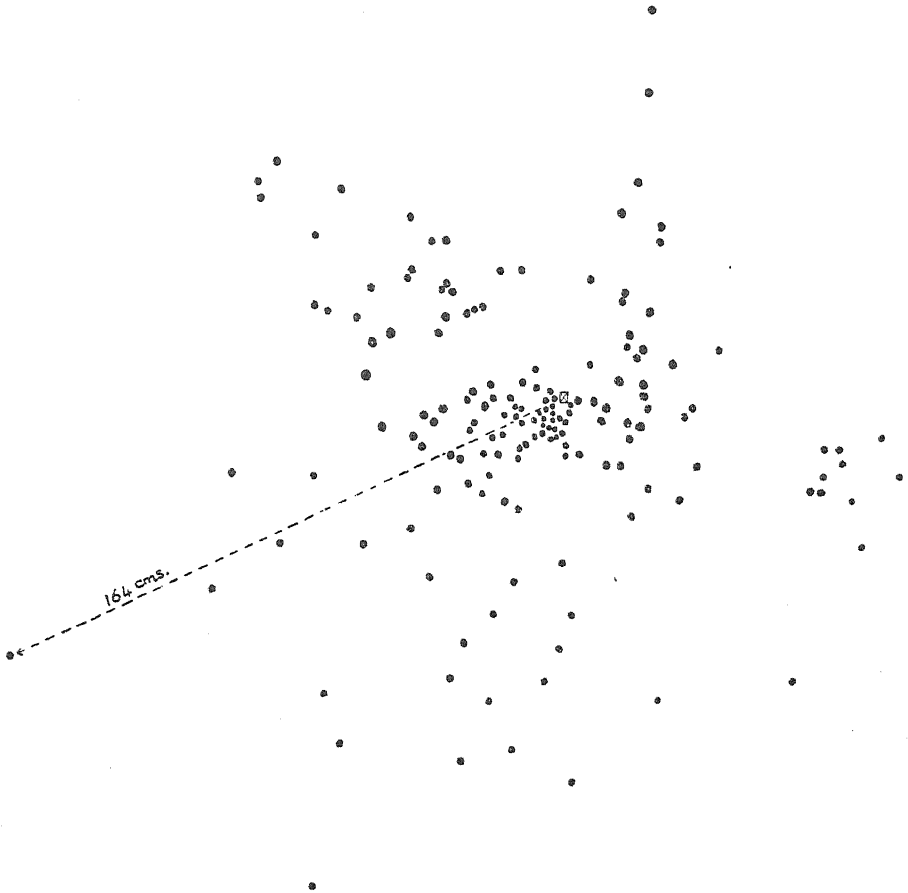
In *Dorotheanthus*, on the other hand, the peduncles when young are long and slender, but after fertilization they curve (Text-fig. 15) until the broad flat base of the capsule rests on the ground. This device for supporting the capsule against the force of impact of the rain is found also in *Carpanthea* (Garside and Lockyer (3)). Further, when the fruit opens, the valves reflex until the tips of the persistent woody stigmas come to rest upon the ground, so that these, possibly, afford some slight assistance in maintaining the horizontal position of the open capsule.



NOTE.

*Carpanthea pomeridiana*, N.E.Br.

As it was possible to make charts of seed dispersal with the apparatus already described, some further experiments were carried out with *Car-*

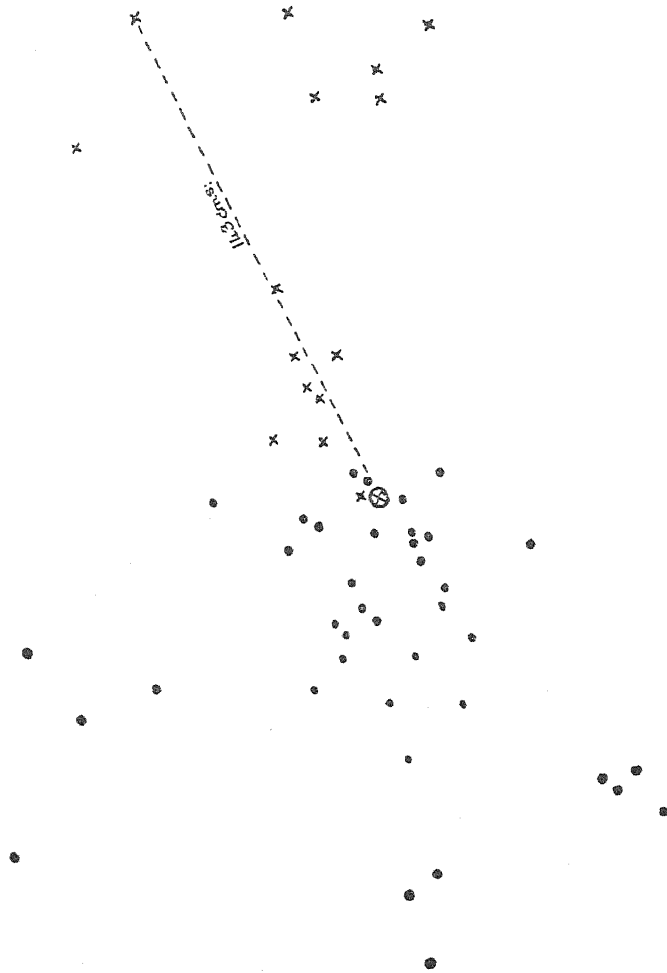


TEXT-FIG. 23. Chart of seed dispersal from an intact capsule of *Dorotheanthus*. Scale 1 in 20.

*panthea pomeridiana*. In a former paper (Garside and Lockyer (3)) it was stated that drops falling on the outside of a capsule ejected seeds through the openings towards the centre of the capsule, and the following experiment confirms this statement.

An open capsule was placed so that the falling drops struck one side of the capsule (i.e. above the middle line, Text-fig. 24). After five minutes the drops were discontinued, the seeds were collected, and their positions marked on the chart with a solid black circle (Text-fig. 24). The drops

were then continued for a further five minutes, but this time arranged to strike the other half of the capsule (i.e. below the middle line, Text-fig. 24). The positions of the seeds ejected during this second period are shown as



TEXT-FIG. 24. Chart of seeds dispersed from a capsule of *Carpanthea pomeridiana*, N.E.Br. For explanation see text. Scale 1 in 20.

crosses (Text-fig. 24). The figure shows that all the drops falling on one side of the capsule eject the seeds across the capsule so that they fall on its opposite side. In other words, a drop falling into the outer opening above a loculus ejects a seed through the inner opening, so that it travels across the centre of the capsule to fall to the ground on the opposite side.

SUMMARY.

1. Hygroscopic fruits of *Bergeranthus scapigerus* and *Dorotheanthus bellidiformis* are described. Both are five-valved capsules which open when made wet by rain but close again on drying.

2. Each valve of *Bergeranthus* possessed two relatively small free hygroscopic keels, the extending bases of which cover part of the inner face of the valve with a single layered hygroscopic skin. In *Dorotheanthus* there are no free keels, but two triangular areas on the inner side of each valve are covered by a four layered hygroscopic skin.

3. In both capsules two superlocular wings extend from the top of each septum to meet corresponding wings from other septa, above the centre line of each loculus. In *Dorotheanthus* the wings extend as far as the ovary wall. In *Bergeranthus* they are less extensive, but the space between the wings and the ovary wall is filled by a large tubercle in each loculus.

4. Experiments are described in which the seeds from both types of capsule are dispersed by falling drops of water.

5. The falling drops strike the wings, and the seeds are splashed from the flooded loculi through the narrow cracks between the superlocular wings.

6. Experiment shows that wings slow down the rate at which the seeds leave the capsule, but at the same time cause a wider and more even distribution of the seeds around the capsule. Charts are given showing the dispersal from an intact capsule and from a capsule the wings of which have been removed.

7. A drop of water (radius 0.269 cm.) falling from a height of two metres ejected a seed of *Bergeranthus* to a distance of 152 cm. The farthest distance that a seed of *Dorotheanthus* was ejected by a similar drop was 164 cm.

8. The capsules of *Bergeranthus* are supported on rigid vertical peduncles. In *Dorotheanthus* the peduncle curves after fertilization so that the broad base of the capsule rests firmly on the ground, thus supporting the capsule against the impact of falling rain. In both cases the capsules are, therefore, suitably orientated for dispersal of their seeds by rain.

In conclusion, I wish to thank Mr. Garside of Bedford College for his valuable help, criticism, and advice in the preparation of this paper. I am also indebted to him for the drawing from which Text-fig. 15 was made. My thanks are also due to Professor Compton and to Mrs. L. Bolus, who kindly sent from the Botanical Gardens, Kirstenbosch, South Africa, the material used in this investigation.

NOTE ON NOMENCLATURE.

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*Dorotheanthus bellidiformis* (Burm. f.) N.E.Br. Möller's Deutsch. Gärtner-Zeit., 400,  
 1928. = *Mesembryanthemum bellidiforme*, Burm. f. Fl. Cap. Prodr. 15 (1768).  
 = *Mesem. criniflorum*, Linn. f. Suppl. Pl. 259 (1781). = *Dorotheanthus criniflorus*,  
 Schwantes.

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4. HUBER, J. A.: Zur Morphologie von *Mesembrianthemum*. Botanisches Archiv, 1924.

EXPLANATION OF PLATE X.

Illustrating Miss Lockyer's paper on Seed Dispersal from Hygroscopic *Mesembryanthemum* Fruits.

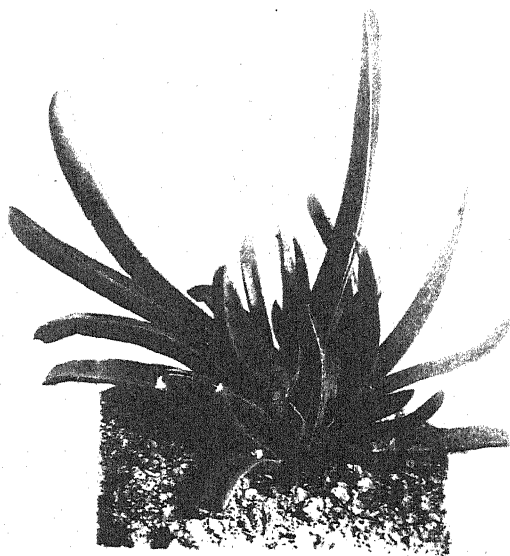
*Bergeranthus scapigerus*.

- Fig. 1. One-year-old plant.  $\times \frac{1}{2}$  approx.  
 Fig. 2. Stalk bearing two fruits.  $\times 1$ .  
 Fig. 3. Seeds.  $\times 3$ .  
 Fig. 4. A closed fruit seen from above.  $\times 3$ .  
 Fig. 5. An open fruit seen from above.  $\times 3$ .

*Dorotheanthus bellidiformis*.

- Fig. 6. Closed fruit.  $\times 3$ .  
 Fig. 7. Open fruit.  $\times 3$ .  
 Fig. 8. Seeds.  $\times 3$ .

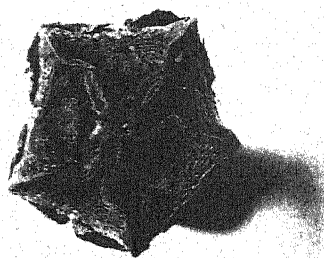




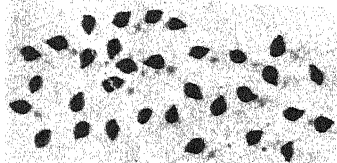
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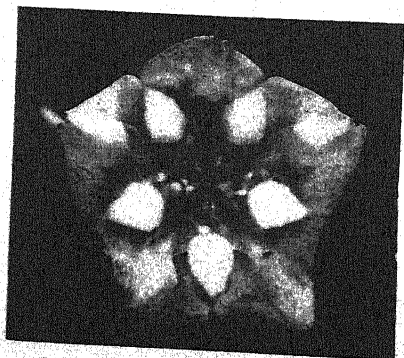
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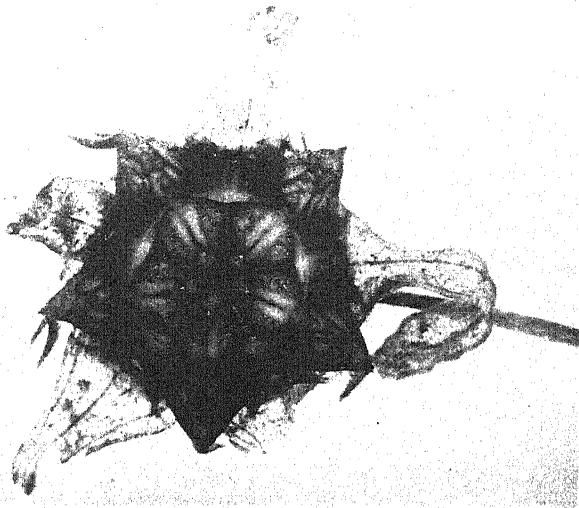


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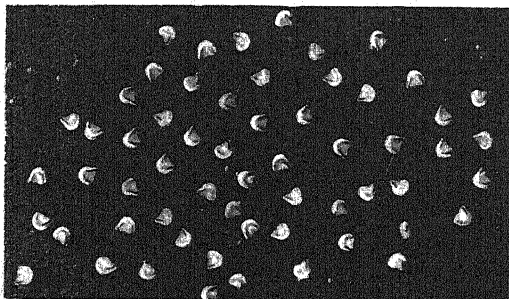
LOCKYER — MESEMBRYANTHEMUM FRUITS.



6



7



8





# A Reversible *Stemphylium*-*Alternaria* Saltation.

BY

S. P. WILTSHIRE.

(Imperial Mycological Institute, Kew.)

With Plate XI and two Figures in the Text.

IN a previous paper (2) the author recorded the saltation of a fungus belonging to the genus *Alternaria* to that of the genus *Stemphylium*. Recently another species of *Alternaria* has been encountered which regularly saltates to a *Stemphylium*, and in this case it has been possible to show that the *Alternaria* can be derived from the *Stemphylium*, from which it appeared to develop in the first instance.

The fungus in question was sent to the Imperial Mycological Institute by Dr. Grimes, of University College, Cork, who isolated it from dairy butter. As received the fungus was clearly a *Stemphylium*, with muriform, non-catenulate spores, which were borne singly, frequently laterally on short branches, or in clusters, and which were oval, rather elongated oblong, cylindrical, or slightly conical in shape, constricted at the septa, with few vertical walls, and with a smooth surface (Text-fig. 1). A number of Petri-dish cultures of the fungus were made on clear maize meal agar, and on one of these bacterial contamination limited the growth of the fungus very considerably. After some weeks' incubation at laboratory temperatures, it was observed that a purplish growth, which on examination under the microscope proved to be that of an *Alternaria*, had grown out from the *Stemphylium* into that part of the agar under the growth-inhibiting influence of the bacterium. It was surmised that saltation had occurred, and transfers of the *Alternaria* to clear maize meal agar in Petri dishes were immediately made. After a time, these showed an apparent saltation to the *Stemphylium* growth at the margins.

Five single *Alternaria* spores were then placed on clear maize meal agar in Petri dishes, one spore to each dish. Four of these spores germinated, and six days later had formed colonies 2.4 to 2.8 cm. in diameter, two of which were saltating to the *Stemphylium* in three places each, and two in one place each. The growth of the saltant was everywhere more rapid than that of the parent. When these single spore cultures were

eight days old, five more single *Alternaria* spores, taken from one dish, were transferred to fresh Dox agar, again one spore to each dish. In this experiment also, one spore did not germinate, but the other four gave colonies which saltated to the *Stemphylium* in each case, the first in two places after six days, the second and third in two and four places respectively after eleven days, the fourth in one place after twelve days. Further replication of these experiments was made on Dox agar with two series of five single spore cultures, and the resulting eight colonies all saltated to the *Stemphylium* within thirteen days (Pl. XI, Fig. 1).

In all the above-mentioned single spore cultures, saltation to *Stemphylium* took place sooner or later, and there is considerable probability that the *Alternaria* invariably saltates to the *Stemphylium* provided it is given facilities for doing so.

The next problem was to find out whether the *Stemphylium* ever saltated to the *Alternaria*. As in the *Alternaria-Stemphylium* saltation previously described (loc. cit.), the more rapid growth of the *Stemphylium* automatically eliminates any saltation to *Alternaria* provided that no special means are taken to ensure its preservation. Two single spore cultures of the *Stemphylium* were therefore made, and small portions of these, two from each, were transferred to clear maize meal agar in four Petri dishes. The next day the plates were inoculated, at a point about 3.5 cm. from the *Stemphylium* transfer, with the bacterium present in the Petri dish in which the original saltation occurred. The delay of a day in inoculating with the bacterium was to allow the surface of the agar to dry up a little and to avoid undue spread of the bacterial colony. The *Stemphylium* rapidly covered the Petri dish except for an area of radius 1.5 to 2.5 cm. under the influence of the bacterium. The edge of the fungus colony in the region of the bacterial growth was well defined, and the mycelium presented the well-known swollen appearance associated with growth under adverse conditions. Two or three weeks after inoculation with the *Stemphylium* a number of hyphae were observed to grow out into the inhibiting area, apparently having become accustomed to the toxic products from the bacterium. On one of the plates there was at one point a much greater development of mycelium than elsewhere, and on examining the culture under the microscope, chains of *Alternaria* conidia on conidiophores arising from this mycelium were plainly visible. The other three plate cultures, however, never showed the *Alternaria* growth.

To confirm this result a series of four Petri dishes containing maize meal agar was inoculated with single spores of the *Stemphylium*, three of which did not germinate, while the fourth developed a healthy colony; after three days' incubation this plate was inoculated with the same bacterium as before. Ten days later a growth resembling the original *Alternaria* saltation occurred in the bacterial zone, and this was subse-

quently confirmed by microscopic examination, when the characteristic chains of *Alternaria* spores were clearly visible (Pl. XI, Fig. 2).

In a repetition of this experiment, five single *Stemphylium* spores each germinated well, and four weeks after inoculation with the bacterium one of the colonies showed what appeared to the naked eye to be an *Alternaria*-like growth, but on microscopic examination this growth proved to be that of the *Stemphylium* and not *Alternaria*. None of the other colonies showed any signs of an *Alternaria* growth.

A similar batch of five single spore cultures of the *Stemphylium* was then made, and again each of these spores germinated well. Inoculation with the bacterium was carried out after one day's incubation, and in two dishes the inoculation was made in the form of a streak. When the cultures were thirteen days old a very clear growth of mycelium, very like that of an *Alternaria* saltation, was observed in the bacterial zone in four of the dishes. After five weeks' incubation the resemblance of these saltant-like growths to the true *Alternaria* saltants was very striking, the long branching mycelium, arising from one point on the margin of the growth-inhibited area and spreading for a distance of 1 or 2 cm. into that area, being indistinguishable macroscopically from that of a real saltant. Microscopic examination, however, failed to show any sign of *Alternaria* spores in any of the dishes, and in some the supposed *Alternaria* growths were found to bear numerous *Stemphylium* conidia. From one of the dishes transfers of sterile mycelium from three *Alternaria*-like growths into the bacterial area were made on clear maize meal agar and from another dish five similar transfers were made. Of the former, two gave *Alternaria* colonies which subsequently saltated to the *Stemphylium*, and one gave *Stemphylium* only. Of the second set of five transfers, each produced *Alternaria* colonies which saltated to the *Stemphylium*.

A further experiment with five single spore cultures on Dox agar yielded in the bacterial zone only *Stemphylium* in each case, except on one dish where two short chains of abnormal spores were seen. No transfers were made from these dishes.

A sixth series of five single spore cultures of the *Stemphylium* was then made and inoculated with bacteria the same day. One of the resulting cultures showed two growths into the bacterial zone. Transfers from one of these gave *Stemphylium* only, while one transfer from the other yielded *Stemphylium* only and a second transfer *Alternaria*, which remained sterile for some time, but saltated to the *Stemphylium*, and finally showed profuse *Alternaria* spore formation over a small area. Another of the single spore cultures of *Stemphylium* gave *Alternaria*-like growths, but transfers from these gave colonies with long, narrow *Stemphylium* spores, which saltated to the original *Stemphylium* very quickly, the angle of the *Stemphylium* sector being much wider than in the case of the *Stemphylium*

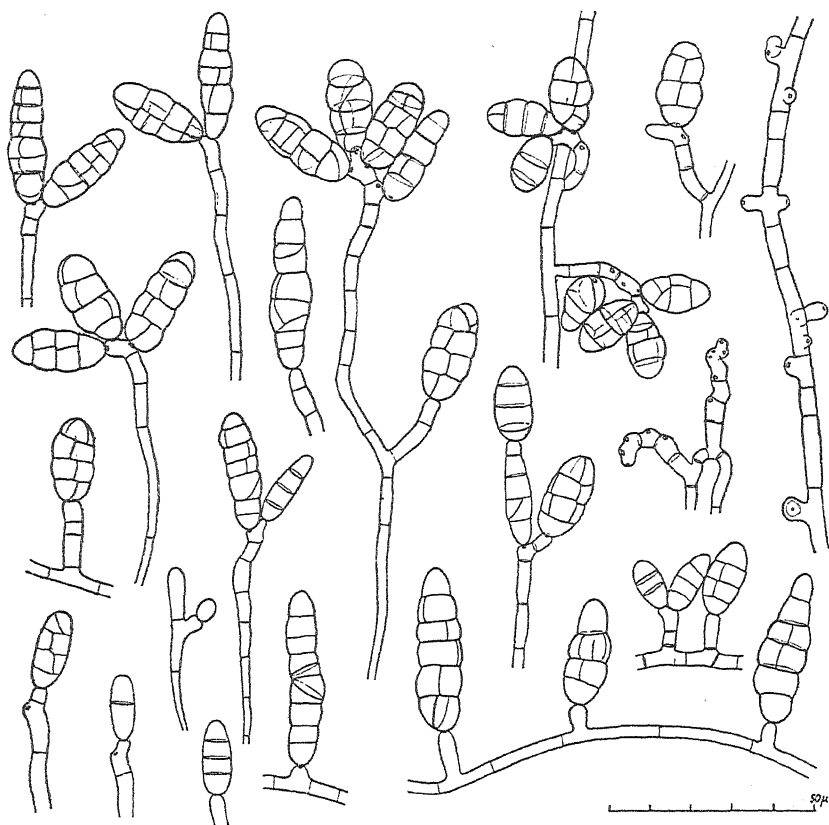
saltant from the *Alternaria*. Subsequently one part of the narrow-spored growth near its limit produced an abundant crop of *Alternaria* spores. Single spore cultures of the narrow-spored saltant gave similar colonies to those from which the spores were taken both in appearance and behaviour.

For the seventh experiment ten transfers were made from two single spore cultures (five from each) to clear maize meal agar in Petri dishes, and inoculations made with the bacterium in straight lines two days later. After twenty-two days' incubation sterile growths into the bacterial zone began to appear, and transfers of these were made to fresh Petri dishes as they were observed. These transfers often remained sterile for a long time, but ultimately an *Alternaria* was obtained from each of the ten *Stemphylium* cultures. The fungus had now been in culture nearly six months, and was evidently beginning to lose its fertile condition. Often only a portion of the *Alternaria* growth showed any development of spores, and this took place frequently where two colonies joined, the powdery appearance of the mycelium clearly indicating the sporiferous area. Sometimes the transfers filled the Petri dish without saltation, and sometimes sporulation occurred long after the saltation had taken place. Two types of *Alternaria* spores were recognizable, an almost cylindrical type and a definitely beaked type, and though Petri dishes seeded with single spores of these types could be distinguished from each other, neither type remained absolutely constant, and it is doubtful whether they represent different saltants. The narrow-spored *Stemphylium* recorded in the previous experiment was also recognized again from one of the transfers.

The conclusions drawn from these experiments show that the *Stemphylium* can saltate to the *Alternaria*, and there is little doubt that the original *Alternaria* was derived from the *Stemphylium* in this way. This saltation becomes apparent when the *Stemphylium* growth is inhibited by the influence of a suitable bacterial colony, and can only be recognized with certainty by observing the formation of the *Alternaria* conidiophores, as the *Stemphylium* may also grow into the bacterial zone after the lapse of some weeks. The *Alternaria* saltant may consist only of sterile mycelium which does not sporulate until transferred to a fresh agar, and sometimes refuses to form spores even then. The *Stemphylium* may also give rise to a narrow-spored saltant, with mostly single spores but occasionally with chains of two, but so far as observed this type of saltant ultimately bears *Alternaria* spores on some part of its mycelium, and reverts to the original *Stemphylium* very rapidly.

The *Stemphylium* fungus grows well on a number of media, but the appearance of the colony varies greatly according to the medium used. On clear maize meal agar, it forms a circular colony rather closely appressed to the substratum. The aerial development of mycelium

is never very marked, but in older cultures a slight darkening in colour indicates the formation of conidia towards the edge of the dish, and not infrequently isolated tufts of 'achromatic variants' were visible on the



TEXT-FIG. 1. Conidia and conidiophores of the *Stemphylium* isolated from butter, which saltates to *Alternaria* under appropriate conditions.  $\times 550$ .

surface of the colony, particularly after the fungus had been cultured for some time. In colour the colony is of a dark olive<sup>1</sup> tint which is very lightly developed and is semi-transparent; the reverse shows no colour difference from the surface view. A distinct zonation is often visible, but this was not due to any special spore development. Spores are borne rather sparingly at first, but ultimately there is a fairly good development, though it is never dense, except perhaps in patches. Many of the spores are borne singly on short lateral conidiophores, some in pairs, threes, or even fives. Very occasionally a chain of two spores may be observed.

On Dox the fungus forms a much denser growth, olivaceous black (1) in colour, with some development of greyish aerial mycelium giving the

<sup>1</sup> Ridgway's colour standards are used throughout this paper.

colony a woolly surface. On the reverse side the colony is also olivaceous black, and the growth is noticeably radiating from the point of inoculation owing to the formation of strands of hyphae. There is a good development of spores, and the mycelium on the surface of the agar is much swollen and dark coloured.

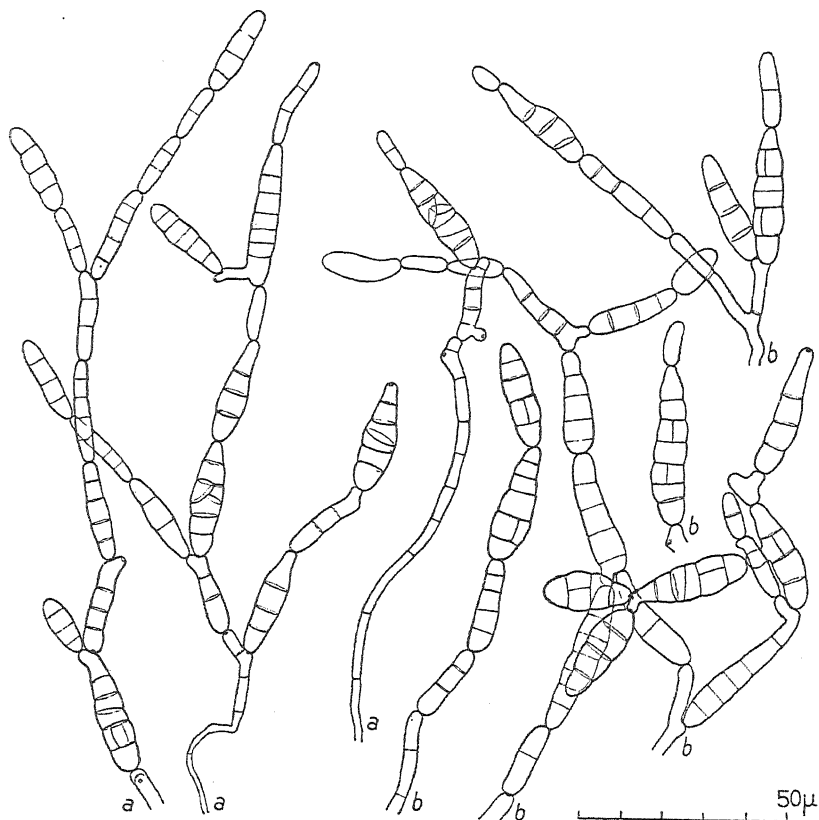
The conidiophores vary much in appearance. In the simplest form, as they occur especially in young colonies, they may be merely short branches borne laterally and perpendicularly on a hypha—often rather swollen and with a terminal scar marking the place where the conidium was attached (Text-fig. 1). There is usually no septum to cut off the conidiophore from the main hypha, and one cell may bear two (or even more) such conidiophores. Older cultures show conidiophores consisting of longer lateral branches, but otherwise similar to those first described, and these are sometimes septate; they may bear up to five or more spore scars, two or three often occurring on the terminal cell of the conidiophore not separated from each other by septa. The conidiophore may also develop from the terminal portion of an ordinary hypha, which, however, is of rather larger diameter and lightly coloured.

The conidium develops as a small oval body on the conidiophore. It elongates to about three or four times its breadth and divides transversely into two cells. Each of these cells may then subdivide again, giving the three transverse walls which can be recognized in many of the spores. The vertical walls develop later. After a conidium has been formed and frequently before it is mature a second is initiated, to be followed perhaps by a third, a fourth, and so on until a cluster is formed; this practice is sometimes so rapid that all the spores are immature and still undergoing development at the same time.

The conidia are oval, elongated oblong, or cylindrical, sometimes slightly tapering towards the apex and constricted at the septa. Very occasionally a conidium of the *Alternaria* type which has borne a second conidium is found. The cross septa number from one to nine, mostly three to five, and the main septa are often more prominent than those formed later. The vertical septa number from none to six or more. The conidia measure 17 to 44 by 8 to 15  $\mu$ . The surface of the conidium is quite smooth. In colour the conidia are dark olive, but they are not opaque. Abnormally large swollen spores occur in colonies adjacent to toxic bacteria where the usual modifications of growth are also apparent.

The *Alternaria* forms a very light greyish colony on clear maize meal agar, often with definite zonation marked by darker growth on the lighter background. The colony is almost transparent and the same colour on the reverse side as on the upper surface. A slight aerial mycelium is developed. In outline the colony is circular and remains so until saltation takes place.

On Dox agar the fungus forms a roughly circular colony, dull greenish-black (2) in colour, shading first to dusky yellowish-green and then to white towards the margin, and with a scanty, floccose, whitish aerial



TEXT-FIG. 2. Conidia and conidiophores of the *Alternaria* which regularly saltates to the *Stemphylium*; *a* = on clear maize meal agar, *b* = on Dox agar.  $\times 550$ .

mycelium. The centre of the colony is powdery with profuse spore development, and is Lincoln green in colour. A characteristic feature of the growth is the dark strands of hyphae which radiate out from the centre to the somewhat feathery margin. On the reverse side the colony is olivaceous black in the centre, shading to dusky olive green in the lighter parts.

The *Alternaria* may saltate when the colony is only 2 cm. in diameter, or saltation may be delayed until the colony almost fills the Petri dish. The saltant starts as a very narrow sector which increases in width very rapidly and bulges in a semicircular fan-like growth beyond the edge of the *Alternaria*. There may be as many as seven saltations of a single colony, and frequently the growth of the *Stemphylium* saltant completely

surrounds the *Alternaria*. The change in colour of the *Alternaria* mycelium to that of the *Stemphylium* is very noticeable on Dox agar, and a loss of the floccose aerial mycelium also accompanies the change.

The *Alternaria* spores (Text-fig. 2) occur in branched chains, and as many as nineteen spores have been counted in a single chain (excluding the spores in the branches). Not infrequently the terminal spore of a chain is more perfectly developed than the rest, and often shows a resemblance to those of the *Stemphylium*. The spores themselves vary from one-celled, small, oblong structures to the large, muriform, obclavate spores typical of *Alternaria*. Mostly, however, the spores are not well developed and lack vertical walls. They are often cylindrical in shape and slightly bent, but may be broader and tapering towards the apex which is marked with a scar, though usually this is almost imperceptible. The best-developed spores are constricted at the septa as in the *Stemphylium*. The surface is smooth and the colour light olive, sometimes almost hyaline. The conidiophores are usually formed from the terminal portions of hyphae; these are slightly enlarged and bear a terminal scar together with one or two secondary scars in some cases. In size the mature spores measure from 10 to 41 by 4 to 13  $\mu$ .

As regards the identity of the species of *Stemphylium* involved, this has not been determined. It is apparently distinct from *S. butyri*, Patterson (1), described from butter in 1900, as the conidia of that species are stated to be verrucose. The general shape of the spores and the manner in which they are borne suggest *S. ericoctonum*, but the spores of this species are distinctly smaller (20 to 28 by 14  $\mu$ ).

In comparing this type of saltation with that described in a previous paper it is evident that they have some features in common. Firstly, in each case, the *Stemphylium* is the more vigorous growth; secondly, both the *Alternaria* forms appear invariably to saltate to *Stemphylium* sooner or later; thirdly, there is a distinct resemblance between the *Alternaria* and its corresponding *Stemphylium* stage, particularly as regards the submerged spores in the saltation previously described and in the shape and smoothness of the spores (which are elongated in both stages, and which, when well developed in the *Alternaria* stage, resemble those of the *Stemphylium*) in the present instance; and lastly, in each case the *Alternaria* appears as the unstable, incompletely developed form, which is probably only derived from the *Stemphylium* under exceptional circumstances.

Some reference was made in the previous paper to the systematic importance of a saltation from one genus to another. In view of the fact that the saltation now described is reversible, it seems quite possible that the earlier one was also, though repeated attempts to induce reversion did not succeed. In these circumstances it seems best tentatively to regard the *Alternaria* saltant as merely a stage in the life-history of the *Stemphylium*,



which normally remains constant, but which may saltate to the *Alternaria* under special conditions. The adoption of this view will avoid any systematic complications and provide a simple solution to an otherwise difficult problem.

#### SUMMARY.

1. A reversible *Stemphylium-Alternaria* saltation is described. The *Stemphylium*, isolated from butter, saltated to the *Alternaria*, and single spore cultures proved (1) that the *Alternaria* regularly saltated to the *Stemphylium*, and (2) that the *Stemphylium* when cultured in proximity to a growth-inhibiting bacterial colony sometimes saltated to the *Alternaria*. Inhibition of growth of the *Stemphylium* was necessary for the *Alternaria* saltation to become apparent, as its rate of growth was much greater.

2. The *Stemphylium* and *Alternaria* forms are described but not identified.

3. The saltation is compared with the *Alternaria-Stemphylium* saltation previously reported, and some general concluding remarks are made.

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#### ADDENDUM.

Since this paper was sent to the press, Miss M. A. Brett has published an account of 'Cyclic saltation in *Stemphylium*' (Trans. Brit. Mycol. Soc., xv. 89-101, 1931), in which she also describes saltation between *Alternaria* and *Stemphylium*.

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1. PATTERSON, F. W.: New Species of Fungi. Bull. Torrey Bot. Club, xxvii. 285, 1900.
2. WILTSHIRE, S. P.: A *Stemphylium* Saltant of an *Alternaria*. Ann. Bot., xliii. 653-62, 1 pl., 4 figs., 1929.

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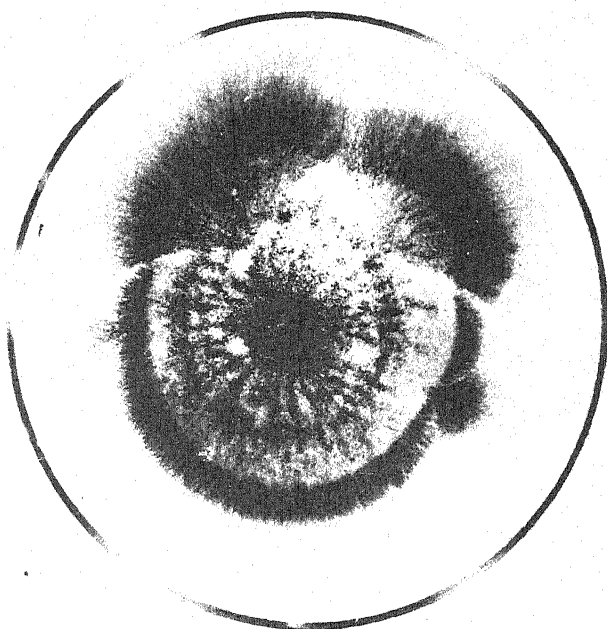
#### EXPLANATION OF PLATE XI.

Illustrating Mr. S. P. Wiltshire's paper on A Reversible *Stemphylium-Alternaria* Saltation.

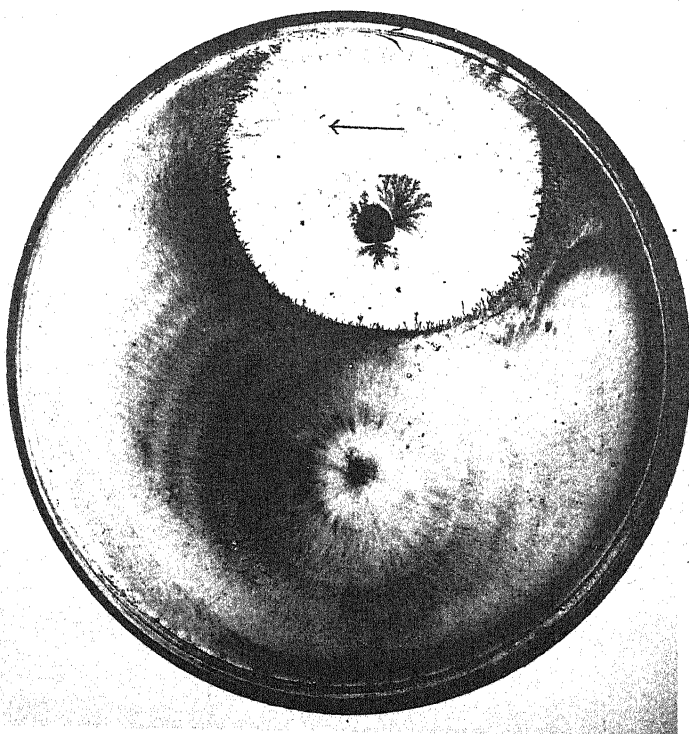
Fig. 1. A single spore culture of the *Alternaria* (derived from a *Stemphylium* isolated from butter) on Dox agar showing saltation to the *Stemphylium* in four places. (Photograph by Mr. G. Atkinson.)  $\times 1$ .

Fig. 2. A culture grown from a single spore of the *Stemphylium* on clear maize meal agar, showing saltation to the *Alternaria*, which can be recognized as a fine and almost imperceptible growth of mycelium from the edge of the circular area under the influence of the growth-inhibiting bacterial inoculation. (Photograph by Mr. G. Atkinson.)  $\times 1$ .





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# On the Identification of Isolated Timber Specimens, with Especial Reference to Fossil Woods.

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With Plate XII.

THE examination of a number of fossil dicotyledonous woods from collections belonging to the British Museum (Natural History), the Stockholm Riksmuseet, and Stockholm University, suggested to the writer of this article that some discussion of the difficulties connected with their identification might form a useful introduction to the consideration of the various fossils, descriptions of which will be given in subsequent papers.

It may be suggested, with all due veneration for the work of the pioneers in the investigation of fossil dicotyledonous woods, that their comparisons of fossil with modern types were often made without a sufficiently wide knowledge of living wood-structures, with the result that generic names were invented which indicated a wholly unwarrantable assumption with regard to the relationships of the specimens to which they were applied; even if the original worker on a particular type, though giving it a somewhat committal generic name, were cautious in his suggestions as to its affinities, it was fatally easy, and indeed not unnatural, for subsequent writers to assume that this generic name meant, *ipso facto*, the inclusion of the type possessing it in a particular family.

A case in point is provided by '*Cornoxydon myricaeforme*'. In 1882 Conwentz instituted the genus *Cornoxydon* for the reception of certain fossil woods from Holstein, possessing a *Cornus*-like structure (7, pp. 157-160); and in 1884 Vater described a petrified wood from the Lower Senonian of Brunswick as being similar in type, not only to the fossil wood *Cornoxydon*, but to the wood of the living *Myrica* also; he therefore adopted the generic name first used by Conwentz, and employed the specific name *myricaeforme* to indicate comparison with *Myrica* (30, p. 846; Pl. XXIX, Figs. 25, 26).

In 1888 Caspary described a wood, probably of Cretaceous age,

[*Annals of Botany*, Vol. XLVI, No. CLXXXII, April, 1932.]

which he termed *Cornus cretacea* (4, p. 39); later this wood was compared, in particular, with that of the living *C. alba* (5, p. 23; Pl. V, Figs. 5-8; Pl. VI, Figs. 1-3); and *C. cretacea* is now included in Edwards's catalogue of fossil dicotyledonous woods as *Cornoxylon cretaceum* (8, p. 29).

Now it should be noted that the genus *Cornus* has a wide distribution in north temperate regions, and contains forty-eight species; according to the 'Age and Area' hypothesis of Willis (31, p. 63), this would indicate that it represents an old type, which it would be not unnatural to find in a fossil condition, even in strata of Cretaceous age.<sup>1</sup> *Myrica* is also a large and widely spread, and therefore presumably old, genus, having forty species in the Northern Hemisphere and the Andes; and it is interesting to note that the secondary wood-structure of the respective families of these two genera is of a very similar *general* type, having a close and fine texture, with numerous small scattered vessels, narrow rays, thick-walled fibres, and comparatively little 'diffuse' wood parenchyma; the wood of the Cornaceae is characterized by the scalariform perforations of its vessels, while the fibres have simple or bordered pits; and that of the Myricaceae shows a general tendency towards the possession of scalariform perforations, though the simple form also occurs, while the fibres have bordered pits (cf. Solereder, 26, vol. i, p. 437, and vol. ii, p. 785).

This same general type of secondary wood-structure, however, is not by any means restricted to the Cornaceae and Myricaceae; it occurs widely throughout the Dicotyledons, as an examination of a large number of sections has demonstrated. A few of the many cases where it is exhibited by *representative* species of certain genera (though not necessarily by the genus as a whole) are tabulated below (Table I<sup>2</sup>); and four examples, one each from the Cornaceae (*Curtisia faginea*), Myricaceae (*Myrica rubra*), Rubiaceae (*Randia* sp.), and Caprifoliaceae (*Viburnum opulus*), are shown in Pl. XII, Figs. 1-4.

It is not intended to imply that the secondary wood of all the types included in this table is precisely similar; the species differ amongst themselves in detail, such as the closeness, depth, and width of the rays, which are, however, generally narrow, being only from 1 to 4 or 5 cells wide; variations also occur in the thickness of the vessel and wood-fibre walls, in the tendency of the vessels to form small groups, or to form more or less distinct pore-zones. It should be noted that these details are liable to

<sup>1</sup> Cf. Berry, 3, p. 241: the oldest known leaves referred to *Cornus* come from the Upper Cretaceous of Greenland and N. America.

<sup>2</sup> Large genera only are given in this Table; the type of wood-structure referred to also occurs in smaller genera, e.g., in *Curtisia* (Cornaceae) with its one species, *C. faginea* (Pl. XII) Fig. 1, and in *Cliftonia* (1 sp.), *Hamamelis* (3 spp.), and *Liquidambar* (4 spp.), mentioned in Table II in comparison with fossil woods of the same type. The present geographical distribution of *Liquidambar* (Mediterranean regions, Asia and N. America), and the discovery of leaves referable to the genus in strata as early as the Upper Eocene of Greenland, Alaska, and Oregon (3, p. 185) are significant.

TABLE I.

*Representative Species of large and widely distributed Genera showing a similar Type of Wood-structure.*

Order.	Family.	Genus.	No. of species in genus.	Distribution of genus.
Myricales	Myricaceae	<i>Myrica</i> , e.g. <i>M. nagi</i>	40	N. Hemisphere and Andes.
Parietales	Theaceae	<i>Eurya</i> , e.g. <i>E. acuminata</i>	40	Mexico, S. America, W. and E. Indies.
		<i>Taonabo</i> , e.g. <i>T. japonica</i>	30	S. America and Asia.
Myrtiflorae	Myrtaceae	<i>Myrtus</i> , e.g. <i>M. bullata</i>	70	Tropical and subtropical regions.
Umbelliflorae	Cornaceae	<i>Cornus</i> , e.g. <i>C. alba</i>	48	N. temperate regions.
Malvales	Tiliaceae	<i>Grewia</i> , e.g. <i>G. populifolia</i>	120	Asia, Africa, Australia, especially tropical regions.
Ericales	Ericaceae	<i>Rhododendron</i> , e.g. <i>R. arboreum</i>	250	Very wide distribution in tropical, temperate, and arctic regions.
		<i>Vaccinium</i> , e.g. <i>V. occidentale</i>	120	N. temperate regions, the Andes, and Madagascar.
Ebenales	Symplocaceae	<i>Symplocos</i> , e.g. <i>S. crataegioides</i>	290	Tropical and subtropical regions.
Rubiales	Rubiaceae	<i>Randia</i> , e.g. <i>R. coriacea</i>	125	Tropical regions.
		<i>Plectronia</i> e.g. <i>P. odorata</i>	100	Palaeotropical regions.
	Caprifoliaceae	<i>Lonicera</i> , e.g. <i>L. alpigena</i>	100	N. temperate regions.
		<i>Viburnum</i> , e.g. <i>V. opulus</i>	110	Temperate and subtropical regions.

variation in individuals of the same species when grown under different conditions. The vessels are generally rather long amongst the types listed in Table I, and while scalariform perforations are the rule, simple and scalariform not infrequently occur side by side, as in some species of *Myrica* and *Vaccinium*. There is also a general tendency for the wood fibres to possess bordered pits, though simple pits also are to be found, as already noted in the case of the Cornaceae.

A similar general type of structure is also possessed by the following fossil woods:

TABLE II.  
Fossil Woods showing a similar Structure to the Woods of the Living Species listed in Table I.

Type.	Horizon.	Area.	References.	Comparisons with other types.
<i>Aptiana radiata</i>	Lower Cretaceous (Apertian)	I. of Wight	Stopes (27, p. 84; Text-figs. 1-5; Pl. VI, Figs. 1, 3, 4, 5; Pl. VII, Fig. 6; Pl. VIII, Figs. 10 and 11) Moll and Janssonius (19, p. 622) Stopes (28, p. 284; Text-figs. 87-92) Bailey (1, p. 448) Felix (10, p. 24; Pl. III, Figs. 2, 3, 4; Pl. IV, Fig. 4) Conwentz (7, p. 157) Vater (30, p. 846; Pl. XXIX, Figs. 25, 26)	Species of <i>Lonicera</i> , <i>Viburnum</i> , <i>Magnolia</i> , <i>Liriodendron</i> ,  <i>Eurya</i> (Ternstroemiaceae = Theaceae), e.g. <i>E. acuminata</i> , also <i>E. glabra</i> and <i>E. japonica</i> . Further comparisons with Aquifoliaceae ( <i>Ilex decidua</i> ) and Cyrillaceae ( <i>Citifonia ligustrina</i> ), <i>Vaccinium</i> , <i>Liquidambar styraciflora</i> .
<i>Liquidambaroxylon</i> , e.g. <i>L. speciosum</i> <i>Cornoxylon</i> , e.g. <i>C. myrtaceiforme</i>	Tertiary	Hungary	Caspary (4, p. 39; 5, p. 23; Pl. V, Figs. 5-8; Pl. VI, Figs. 1-3) Krausel and Schönfeld (15, p. 277; Text-figs. 20-6) (15, p. 282)	<i>Cornus</i> , <i>Cornoxylon</i> (as defined by Conwentz) and <i>Myrica</i> ; Vater also notes that <i>Deutzia</i> , <i>Philadelphus</i> , and <i>Liquidambar</i> show similar structure to <i>Cornus</i> . <i>Cornus alba</i> . (Other forms showing a similar type of structure are mentioned, viz. <i>Deutzia</i> , <i>Philadelphus</i> , <i>Myrica</i> , <i>Liquidambar</i> , <i>Hamamelis</i> , &c.), <i>Cornoxylon</i> .
<i>C. cretaceum</i>	Lower Cretaceous (L. Senonian) Probably Cretaceous	Brunswick		May be the young wood of <i>Cornoxylon latiporosum</i> ; compared with <i>Hamamelis</i> and <i>Liquidambar</i> .
<i>C. latiporosum</i>	Miocene	S. Limburg		<i>Eurya latifolia</i> .
<i>C. sp.</i>	Miocene	S. Limburg		Compared with recent species of <i>Vaccinium</i> . Attention is drawn to the close structural resemblance between the woods of <i>Viburnum</i> and <i>Cornus</i> . <sup>1</sup>
<i>Ternstroemiacinium</i> , e.g. <i>T. euryoides</i> <i>Vaccinium</i> sp. <i>Viburnum</i> sp.	Eocene  Pleistocene Prehistoric (Loess)	Caucasus  Poland Czechoslovakia	Felix (11, p. 100; Pl. X, Fig. 4.) Schönfeld (25, p. 124) Szafer (29, p. 349) Fietz (12, p. 420)	

<sup>1</sup> The similarity between the wood-structure of *Viburnum* and that of *Cornus* is illustrated by Macbride's original identification of a new *Viburnum* (*V. peruvianum*) as *Cornus peruviana* (17, p. 29).



As amongst the recent forms, scalariform perforations appear to be general in these fossils also (cf. *Aptiana radiata* (27, pp. 85, 86; Text-fig. 2) and *Cornoxydon* spp. (e.g. *C. latiporosum*, 15, pp. 277 *et seq.*)); bordered pits are very distinct on some of the wood-fibre walls in *A. radiata* (27, pp. 85, 86; Text-fig. 1; Pl. VIII, Fig. 10), and they were also noted by Conwentz in his original description of *Cornoxydon Holsatiae* (7, p. 158); but details of this kind are naturally often difficult to determine in fossil material, their demonstration being dependent upon its state of preservation.

Tables I and II clearly indicate that the wood structure of the fossil *C. myricaeforme* represents an old and widely-ranging type. In the absence of associated remains of leaves, buds and twigs, flowers or fruits, it might therefore be compared with a considerable number of living forms other than *Cornus* and *Myrica*; and it is unfortunate that it possesses a generic name which implies affinity especially with *Cornus*, for it has very naturally found its way, together with six other 'species' of *Cornoxydon*, equally suspect with regard to their real systematic position, into the Cornaceae (8, p. 83).<sup>1</sup>

The type of secondary wood-structure exhibited by the recent and fossil forms reviewed above is not only old and widely distributed in the Dicotyledons, but it may also be described as a *generalized type*, for it has *no particular distinguishing features* such as zoning of the xylem parenchyma, definite arrangement of the vessels, or 'storied' disposition of the rays, parenchyma, and fibres (as seen in tangential section of the wood).<sup>2</sup> It is evident that, in the absence of distinctive associated remains of leaves or other vegetative or reproductive organs, any fossil showing this old and generalized type of wood-structure would be difficult to place systematically.

It is now necessary to examine a representative case where some immediately outstanding feature of structure occurs; the alternative zoning of wood parenchyma and fibres, termed by W. S. Jones the '*Ficus-type*'

<sup>1</sup> An instance of misinterpretation of comparisons occurred in the case of certain fossils to be described in the next paper of this series. In 1914 the writer examined the fossils, made notes of their structure, and compared each one with various living types. In no case was a name given, or a definite suggestion of affinities made, because, on the one hand, the structure of the fossils is not sufficiently good to provide certain necessary data, and, on the other hand, a sufficiently wide range of living wood-structures was not available for comparison. In summarizing the notes sent to him, however, Dr. Oswald makes the following statement: 'the specimens represent types belonging to the Malvaceae (similar to the African *Bombax insigne*), to the Geraniales (*Humiria*), to the Papilionales, and to the Caprifoliaceae (similar to some species of *Lonicera* and *Viburnum*)' (21, p. 130). Such a statement is wholly unjustifiable in view of the cautious comparisons originally made by the writer, and maintained, after a very much extended examination of recent wood-structures.

<sup>2</sup> The distribution of this type of structure in space and time (Tables I and II), and its 'generalized' character, indicate that it may be primitive amongst Angiosperms; it is therefore interesting to note that Bailey reaches a similar conclusion from his studies on cambial growth and development (1, pp. 442-4; see also 2).

of structure (14, p. 101; Figs. 125, 126), will serve as an instance (Table III).

The *Ficus*-type of structure is shown by the above table to be very common amongst dicotyledonous families;<sup>1</sup> it also appears to be an old type, since it occurs in certain large genera with wide geographical distribution, for example, amongst the Leguminosae, Guttiferae, and Sterculiaceae (cf. Table III).

As in the case of the species listed in Table I, those in Table III, whilst showing the same general type of structure, differ amongst themselves in detail: for example, the width and depth of the rays, their homo- or heterogeneous character, their tendency to increase in width in traversing the parenchyma zones; the inclusion of the vessels entirely within the parenchyma, or their partial independence of it; the proportional width of the parenchyma and fibre zones; and the storied or non-storied arrangement of the rays, parenchyma, and fibres.

It is clear that a very thorough knowledge of the many combinations which these variable characters may form in dicotyledonous woods is necessary before any attempt can be made to place systematically any specimen, recent or fossil, showing the *Ficus*-type of structure, by an examination of a portion of its wood alone. In the literature relating to fossil woods, there are frequent references to Nordlinger's 'Querschnitte' (20); but in the comparison of specimens, not only should transverse sections be examined, but longitudinal—particularly tangential—sections also. A comparison which was made, during the course of these investigations, between a specimen of *Cola nitida* (Sterculiaceae) from West Africa, and one of *Symphonia globulifera* (Guttiferae) from South America, will serve to illustrate this point.

These two specimens were found to possess the *Ficus*-type of structure, presenting, in transverse section, a very similar general appearance (Pl. XII, Figs. 5 and 6). In the material available, it is true, the dimensions of the cells in *Cola* are distinctly smaller than in *Symphonia*, the vessels are less numerous, and the relative amount of mechanical tissue is somewhat greater; but these are points which may for the moment be left out of account, for they are subject to variation in different regions of the same tree, and it is not possible to say whether the sections of *Cola* and *Symphonia* available for examination were taken from comparable regions of their respective trees.<sup>2</sup>

A comparison of the two photographs in Pl. XII, Figs. 5 and 6, however, will show that the parenchyma bands are from 3 to 5 or 6 cells wide in

<sup>1</sup> Gamble (13, p. xvi) also notes the common occurrence of 'concentric lines of soft texture'.

<sup>2</sup> They are very common in the Leguminosae. . . . They also occur in *Garcinia* and *Mesua* among Guttiferae; in *Elaeodendron*, *Celastrus*, and *Lophopetalum* among Celastrineae; in *Heynea*, *Amoora*, and *Walsura* among Meliaceae; *Cordia* in Boraginaceae; in *Ficus* and in other genera.

<sup>2</sup> Cf. Clarke's researches on the wood of the Elm, to which reference is made on p. 362.

TABLE III.

*Representative Species of Genera in which the 'Ficus-type' of Wood-structure occurs.*

Order.	Family.	Genus.	No. of species in genus.	Distribution of genus.
Urticales	Moraceae	<i>Ficus</i> , e.g. <i>F. Ben-jamina</i>	700	Tropical regions, chiefly in India and Polynesia.
Ranales	Anonaceae	<i>Xylofia</i> , e.g. <i>X. Eminii</i>	60	Tropical regions.
Rosales	Leguminosae	<i>Pterocarpus</i> , e.g. <i>P. marsupium</i>	24	Tropical regions.
		<i>Baphia</i> , e.g. <i>B. nitida</i>	12	Tropical Africa and Madagascar.
		<i>Cynometra</i> , e.g. <i>C. Alexandri</i>	30	Tropical regions.
		<i>Pongamia</i> , e.g. <i>P. glabra</i> (and many others of the Leguminosae)	1	India, Burma, Ceylon.
"	Saxifragaceae	<i>Brexia</i> , e.g. <i>B. spinosa</i>	1	Madagascar, Seychelles.
Sapindales	Sapindaceae	<i>Melicocca</i> , e.g. <i>M. bijuga</i>	2	Tropical America and W. Indies.
	Celastraceae	<i>Celastrus</i> , e.g. <i>C. rhombifolius</i>	30	Tropical and subtropical regions.
Parietales	Ochnaceae	<i>Lophira</i> , e.g. <i>L. procera</i>	2	Tropical Africa.
"	Violaceae	<i>Rinorea</i> , e.g. <i>R. cibbiensis</i>	60	Tropical regions.
"	Guttiferae	<i>Symphonia</i> , e.g. <i>S. fasciculata</i>	6	Tropical America, Africa, and Madagascar.
		<i>Rheedia</i> , e.g. <i>R. Laka</i>	17	Tropical America and Madagascar.
		<i>Garcinia</i> , e.g. <i>G. gowa</i> (and other Guttiferae)	200	Palaeotropical regions.
Malvales	Sterculiaceae	<i>Cola</i> , e.g. <i>C. nitida</i>	50	Africa.
		<i>Sterculia</i> , e.g. <i>S. elegantifolia</i> <sup>1</sup>	100	Tropical regions.
Ebenales	Sapotaceae	<i>Sideroxylon</i> , e.g. <i>S. brevipes</i>	90	Palaeotropical regions.
Tubiflorae	Boraginaceae	<i>Ehretia</i> , e.g. <i>E. staudtii</i>	40	Tropical regions, chiefly in the E. Hemisphere.

<sup>1</sup> It should be noted that while certain species of *Sterculia* show the 'Ficus-structure', the genus as a whole presents a very wide range of structural type.

*C. nitida*, and from 4 to 6 cells wide in *S. globulifera*, the cells being radially arranged in each case; the zones vary from about the same width as the intervening fibre bands to roughly half their width in *C. nitida*; while they are a little wider in proportion in *S. globulifera*. The vessels are scattered, and typically occur singly or in pairs in each case, and are not necessarily completely enclosed in the parenchyma zones. The rays are from 1 to 5 or 6 cells wide in *C. nitida*, from 1 to 4 or 5 in *S. globulifera*; they are numerous, slightly more so in the latter than in the former case; they enlarge a little in the parenchyma zones, and show a certain degree of variability in the size of their cells.

Apart from the difference in the size of the elements generally, the main difference between the transverse sections of the two species (as here represented) consists in the somewhat greater regularity in the radial arrangement of the wood fibres in *S. globulifera*, a difference which appears to be correlated with a smaller amount of variation in their size; this seems, therefore, to be a difference in degree rather than in kind. Further, the parenchyma zones in *C. nitida* are on the whole less undulating than in *S. globulifera*; it is possible, however, that this difference is correlated simply with the smaller number of vessels in *C. nitida*, for the vessels certainly have a rather disturbing effect on the contours of the zones.

It is clear that the main diagnostic characters, as revealed by transverse sections of the two woods—i.e. the regular alternation of the parenchyma and fibre bands; the distribution and arrangement of the vessels, and their relation to parenchyma and fibres; the numerous and comparatively narrow rays, with cells of variable size, and a slight increase in width within the parenchyma bands—indicate that they are of the same general type; the transverse sections do not appear to present any *fundamental* difference between them. In tangential section, however, the two species are immediately distinguishable (Pl. XII, Figs. 7 and 8), owing to a difference in the arrangement of the parenchyma cells; for in *C. nitida*, this is regular and 'storied' (as in the Sterculiaceae generally), while in *S. globulifera* there is no trace of the storied structure, the disposition of the parenchyma cells being irregular. This difference in the arrangement of the elements is *fundamental*, for it is due to a difference in the growth and behaviour of the cambium cells themselves (cf. 1, p. 443); in the storied arrangement, these tend to lie in parallel horizontal series, the sliding growth, which produces the irregular arrangement, having been eliminated.

The storied elements of *C. nitida*, therefore, constitute a distinct and cogent difference between this species and *S. globulifera*; but, for the demonstration of this difference, reference must be made to tangential sections of the wood.

The storied arrangement of parenchyma, and, in some cases, of rays

and fibres also, occurs in combination with the *Ficus*-structure in other types than *C. nitida*: in many of the Leguminosae, for example, and also in *Ficus* itself, although, in this genus, storying is apparently somewhat sporadic, and may or may not be definitely observed in a small section of the wood. Cambial growth is evidently in a transitional condition in *Ficus*, the arrangement of its cells in parallel horizontal series not being fixed.

Various fossil dicotyledonous woods showing the *Ficus*-type of structure have been referred to '*Ficoxylon*', for example, *F. tropicum*, from Tertiary strata of Bohemia (9, p. 81), and *F. cretaceum*, from the Oligocene of Egypt (23, p. 14; Pl. V, Figs. 17-19). *F. tropicum* has been compared with *F. cordata* (itself an ill-defined and composite species), and *F. cretaceum* with *F. Sycomorus*; but it is clear that on the evidence of wood sections alone, the 'genus' *Ficoxylon* must be accepted with reserve as indicating that the fossils bearing it possess a particular type of structure rather than that they have a definite systematic relationship.

Moll and Janssonius, during the course of their investigations on the timbers of Java, have become convinced that the families of Dicotyledons are definitely characterized by their wood-structures (18 and 19). Since all their material was obtained from a restricted area, the types which they studied might quite well lead to such a conclusion.<sup>1</sup> It is also doubtful whether Moll and Janssonius examined representative sections from all parts of any one tree in order to obtain an idea of *essential* points of structure.<sup>2</sup> The necessity of so doing is amply indicated by Clark's recent report on the anatomical structure and physical properties of the Elm (6). In this report it is noted that 'in any one annual ring, the elements increase in dimensions from below upwards, until a maximum is reached, after which they decrease in size. At a given height in the tree the elements of successive annual rings increase in dimensions until a certain size is reached, and then fluctuations occur which are probably due to the variations in external conditions. In ring-porous hardwoods the wood produced in later life is generally more porous and has a smaller proportion of mechanical tissue than that formed during youth.

'The number and type of rays produced in a species seem to depend largely upon inherited factors and age; the total ray volume is closely related to environment, conditions allowing greater metabolism apparently demanding greater ray volumes for storage purposes. . . .'

<sup>1</sup> Cf. Stopes's comment on these authors' account of the Javanese Magnoliaceae: 'a very different account . . . from the one that would have been written had the woods from temperate regions and not those from Java been the main object of their study' (27, p. 90).

<sup>2</sup> Cf. Pritchard and Bailey (22, p. 669), who remark upon the desirability of examining sufficient material of each species to be certain of the limits of variability of each diagnostic character.

'Wood from near the centre of the tree differs considerably from that formed in later life' (6, p. 1).<sup>1</sup>

Gamble, in his work on Indian Timbers, notes that while it is true that some families and genera have woods of similar character and structure, as in the case of the Anonaceae, the species of which are generally characterized by regular, ladder-like, concentric bars of parenchyma on a transverse section of the timber, there is no fixed rule for determining families and genera by means of their wood; in some cases, indeed, the woods of different species even of the same genus are very divergent in character, as in the species of *Dalbergia* (Leguminosae) (13, p. xvi).

Further evidence against the opinion of Moll and Janssonius is provided by the recent extended researches of Kribs on material from various sources, belonging to the Meliaceae. These show that there is so great variation in both physical and anatomical characters in this family that it is not possible 'to find a set of characters which will distinguish the Meliaceae, as a whole, from other dicotyledonous families' (16, p. 736).

Moreover, during an examination of many hundreds of transverse and longitudinal sections of recent timbers, it has become increasingly evident to the writer of this paper that wood from the *same species* varies in details of structure under different conditions of soil and climate; and that, as Clarke's work on the Elm shows, wood of the *same tree* may vary with age, position in the tree, or change of external conditions. It may be, of course, that as a systematic study of the woods of species, genera, and families progresses, essential and unifying details of structure will be discovered; it may be that, as yet, too little is known of minute structure and its exact significance, to indicate what is important systematically, and details which are determinative in one group may be of no importance in another. The systematic study of wood-structures resolves itself into the finding of constant and invariable sets of anatomical characters in any one type.<sup>2</sup> This, of course, entails much work on authentically named material from all possible sources, from all parts of a plant, of all ages, and produced under various conditions of growth—a laborious undertaking. Clarke's work on the Elm, however, indicates that in the three species (*Ulmus campestris*, *U. montana*, and *U. major*) investigated by him, the dimensions of the wood elements formed in the adult period are of specific diagnostic value; also 'in closely related species it is probable that the type and dimensions of elements are of greater diagnostic value than the proportions in which they are present' (6, p. 22). Coherent and confirma-

<sup>1</sup> The photographic plates appended to Clarke's report show variations in structure which are of great interest. *Rate of growth* as affecting structure is illustrated in Pl. V; varying structure on *different radii* (north, south, east, and west) of the *same annual ring*, in Pl. VII; the frontispiece shows the difference in structure between *youthful stages* (in the centre of the tree) and later or *adult stages* (when the elements have reached full size).

<sup>2</sup> Cf. Bailey, 1, p. 446.

tory results in a certain number of cases may considerably lessen the labour of systematic wood-structure investigations by providing clues (1) as to the *essential* points to be sought for in compiling sets of anatomical characters for diagnostic purposes; and (2) as to the particular region of the plant in which these essential characters may be found.

That the necessary information for the systematic diagnosis of wood-structures may be at length forthcoming is an ideal to hope for. Moll and Janssonius may eventually prove to be correct in their view that a specimen may be placed systematically on its wood-structure alone; for the moment, however, workers on recent timbers are advisedly very cautious in their identification of isolated specimens of wood; in a debatable case, material would be referred to a *type* of structure rather than to a genus or even family. Why, then, should palaeobotanists be more adventurous in dealing with their material, which may consist of only a small piece of fossilized wood, often too imperfectly preserved to supply adequate data? Moreover, investigators of fossil dicotyledonous woods—even those woods derived from Tertiary strata—are not *necessarily* dealing with types whose combinations of anatomical details are in existence at the present day.

In 1912 Stopes exercised great caution in naming and comparing her Cretaceous Angiosperms, giving generic names suggestive of horizon and locality rather than of possible affinities: for example, *Aptiana* (from the Aptian strata of the Isle of Wight), and *Woburnia* (from Woburn Sands).<sup>1</sup>

The present writer, however, is in agreement with Mr. Edwards that it is undesirable to multiply generic names to describe these fossil woods (8, p. 4). Bailey advocates the prefixing of 'para' to an existing generic name in order to indicate structural peculiarities and putative genetic affinities (1, p. 448). This also seems undesirable, for, in many cases, at least, any *generic* use of the name of an existing genus, even in combination with a prefix or affix, may lead to unjustifiable assumptions of affinity.

In the writer's subsequent descriptions of fossil woods, Edwards's suggestion will be adopted, namely, that the old generic name of Schleiden—*Dryoxylon*—should be used where the affinities of the specimen are uncertain (cf. 24, p. 28); in some cases a specific name suggesting *similarity* with a recent genus will be appended, but it cannot be too strongly insisted that this specific name does not entitle its possessor to a place in the natural system; the 'species' must remain *incertae sedis* in the absence of associated vegetative and reproductive parts which would give further clues as to affinities. In other cases, where it is impossible to decide upon any one definite comparison amongst many possibilities, it is deemed advisable to give a specific name descriptive merely of the horizon or locality from which the specimen was obtained; or, in some instances, horizon and

<sup>1</sup> See Stopes (27) and (28). *Woburnia porosa* has now been transferred to *Dipterocarpoxydon* by Kräusel (see 8, pp. 35 and 82).

locality may be combined in the specific designation, in order to provide a more complete means of reference to the specimen.

The grateful acknowledgements of the writer are due to Dr. L. Chalk for his courtesy in giving every facility for work in the Wood-structure Laboratory of the School of Forestry, Oxford; and to Mr. A. L. Clinkard, also of the School of Forestry, for the photographs used in illustration of this paper.

October, 1931.

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## EXPLANATION OF PLATE XII.

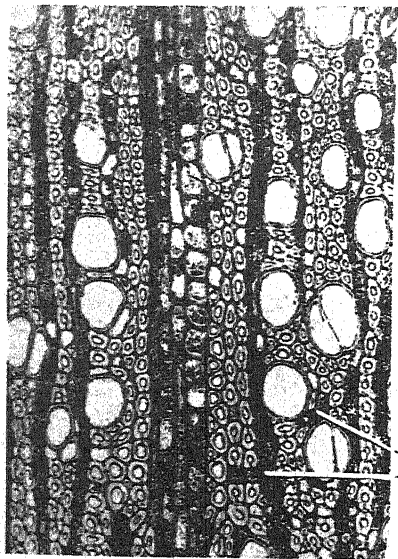
Illustrating Miss Bancroft's paper On the Identification of Isolated Timber Specimens.

All figures are from photomicrographs.

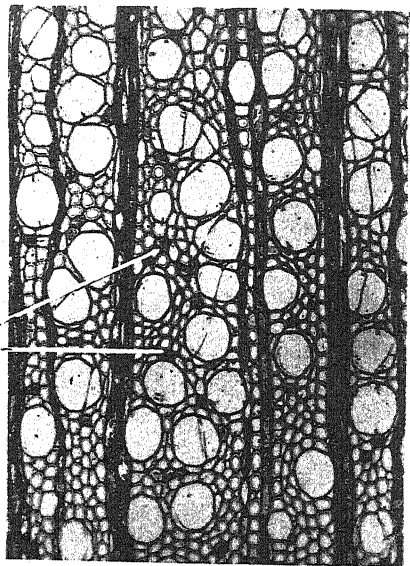
Figs. 1-4. Examples of wood-structure (transverse sections) to illustrate the 'generalized type' described in the text. Fig. 1, *Curtisia faginea* (Cornaceae), Fig. 2, *Myrica rubra* (Myricaceae), Fig. 3, *Randia* sp. (Rubiaceae), Fig. 4, *Viburnum opulus* (Caprifoliaceae). The texture of the wood is close and fine in each case, the magnification being 100 diameters. Note the numerous small, scattered vessels, arranged, typically, singly or in pairs; the thick-walled fibres; the small amount, and scattered distribution, of the xylem parenchyma (*p.*); and the narrow rays.

Figs. 5 and 6. Transverse sections of the wood of *Cola nitida* and *Symphonia globulifera* respectively.  $\times 80$ . Note in each case the regular alternation of parenchyma and fibre bands; the vessels, arranged singly or in pairs; the relation of the vessels to fibre and parenchyma; and the rays with cells of variable size, increasing slightly in width within the parenchyma bands.

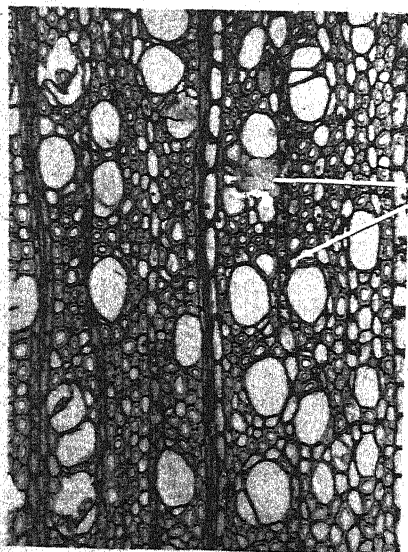
Figs. 7 and 8. Tangential sections of the wood of *Cola nitida* and *Symphonia globulifera* respectively.  $\times 80$ . Note in *Cola* the regular, storied arrangement of the parenchyma, in definite contrast with its irregular arrangement in *Symphonia*.



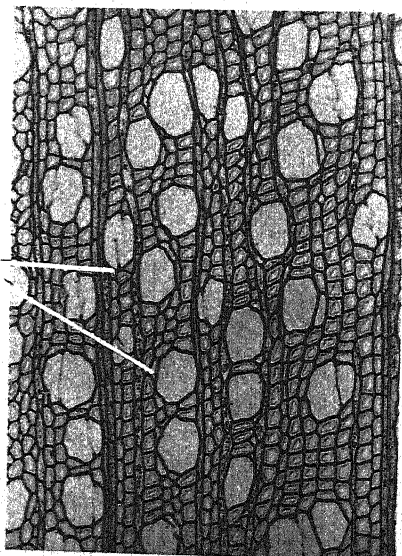
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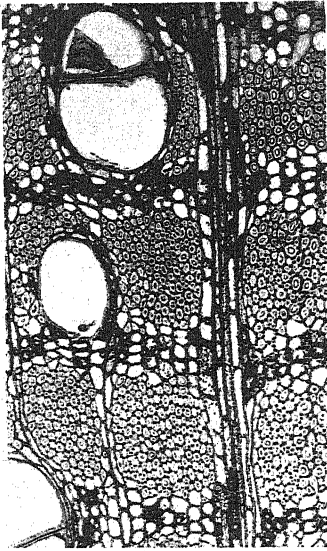
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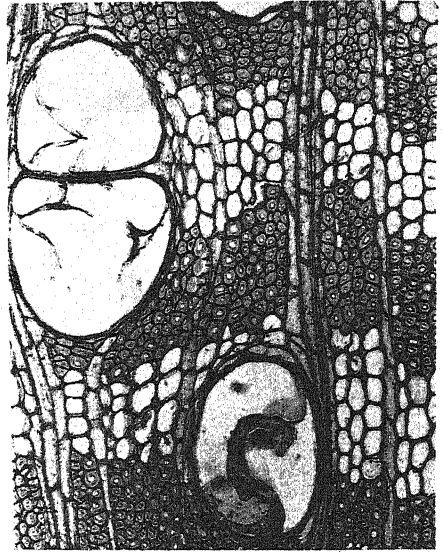
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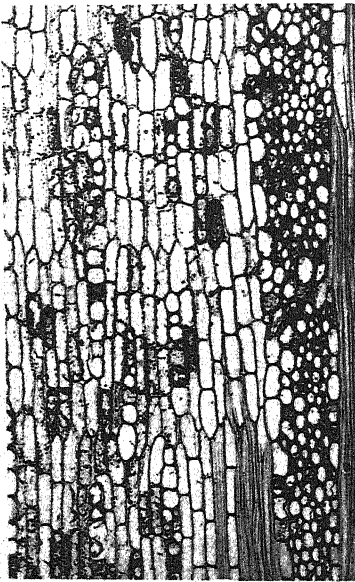
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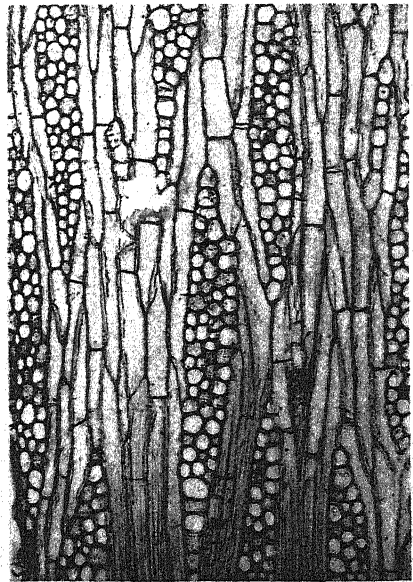
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# Physiological Studies in Plant Nutrition.

## III. Further Studies of the Effect of Potash Deficiency on the Rate of Respiration in Leaves of Barley.

BY

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With seven Figures in the Text.

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### INTRODUCTION.

IN a previous paper (5) the results of some experiments on the respiration and assimilation rates of leaves of barley grown under different manurial treatments were presented. It was there shown that, over a fairly wide range of manuring, phosphate has very little effect on the respiration rate, a deficiency of nitrate reduces respiratory activity, while potash deficiency increases it markedly. It was suggested (5, p. 154) that respiration rate must be a function of the amount of protoplasm, which in its turn must depend on the supply of nitrogen; hence, when this element is in the minimum, respiration rate, at least as ordinarily measured, is lowered. No evidence was found of a direct relationship between respiration rate and potash, and it was assumed that the increase in the rate under potash deficiency is due to the potash concentration determining the level at which other constituents of the cell are maintained.

The present paper is the outcome of similar experiments performed in the summer of 1928, designed to elucidate further the relationship between potash and respiration.

#### EXPERIMENTAL PROCEDURE.

The experimental procedure was similar to that of the previous year; barley of the variety Plumage Archer was used, and the plants were grown three in a pot in sand culture as before. Four nutrient solutions were employed, which differed only in the amount of potassium sulphate they contained; the following are the weights of the pure salts given to each pot in the 'fully manured' series:

$\text{Na}_2\text{HPO}_4$	2.52	gram.
$\text{NaNO}_3$	9.1	"
$\text{K}_2\text{SO}_4$	1.85	"
$\text{CaCl}_2$	0.37	"
$\text{MgSO}_4$	0.61	"

This solution is identical with the one given to the 'fully manured' series of 1927. The plants grown under these conditions will subsequently be referred to as 'Series A'. Series 'B' and 'C' differed in having only one-fifth and one-tenth respectively of the amount of  $\text{K}_2\text{SO}_4$  given to Series A (0.37 and 0.185 gram.), while Series 'D' was grown without the addition of any potash, except such as was present in the tap-water used for watering the pots.

The seed, previously sterilised with formalin, was sown on May 2, and the nutrients applied on May 7; germination began on May 10, and was practically completed by May 12.

The technique used in 1928 was almost identical with that of 1927, but instead of working in a constant temperature room, the katharometer and leaf chamber were housed in a large thermostat.

A higher temperature was used than in the previous year; the maximum variation between different experiments was  $2^\circ\text{C}$ ., and the observed rates have been given a slight correction so as to express them all at the approximate mean of  $24^\circ\text{C}$ . Owing to the higher temperature, the respiration rates were at a considerably higher level than those of the previous year, and therefore the 'zero drift' of the instrument was of relatively less importance; the maximum error in the respiration rate which is ascribable to such a cause cannot be greater than 2 per cent.

Respiration rates of one particular leaf from each of the four series were determined each week, the determinations occupying four successive days. The first determinations were made on the first leaves from May 23-26, but owing to an accident the results from Series B and D were untrustworthy. The succeeding eleven weeks provided respiration rates

from the successive young leaves on the main stem as they reached maturity, from the second to the tenth. Because of differences in the rates of emergence from weekly intervals, the seventh leaf was omitted, while

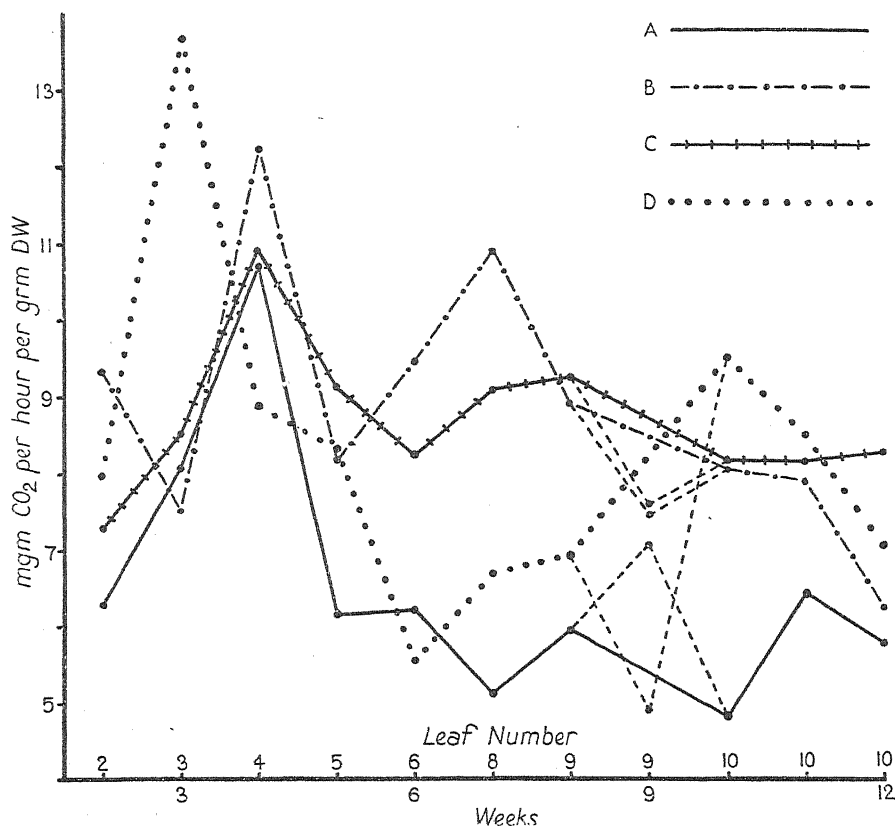


FIG. 1. Graph showing rates of respiration, on a dry weight basis, of the successive leaves from the four potash series.

two successive determinations were made on the ninth. The last three determinations were made on the tenth leaf, and show the senescent changes thereof.

In actual practice each observed rate was that given by at least two leaves cut from different plants, strips about  $3\frac{1}{2}$  in. long being taken from the central region. The cut ends of the leaves dipped into a film of water.

## EXPERIMENTAL RESULTS.

### *Respiration Rate and Potash Deficiency.*

In Table I are presented the rates of respiration, corrected to the approximately mean temperature of  $24^{\circ}$  C., and expressed in terms of dry

weight and leaf area. These values are plotted in Figs. 1-4. In Figs. 1 and 2, respiration rate is plotted for the four potash concentrations against time, whereas in Figs. 3 and 4 the corresponding weekly rates from the

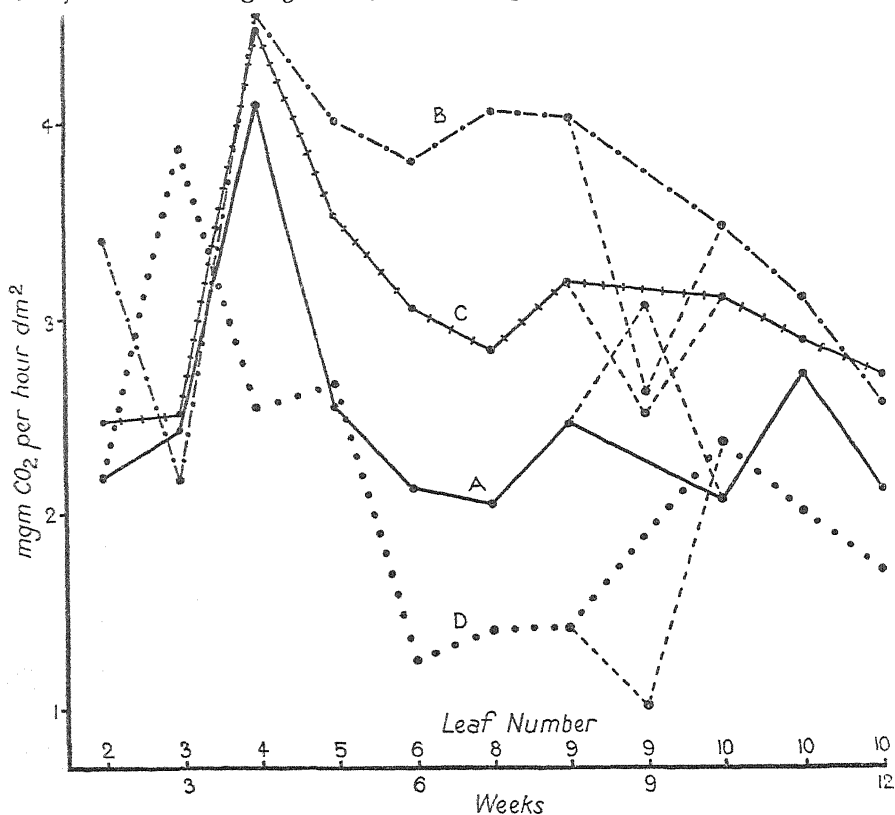


FIG. 2. Graph showing rates of respiration, on a leaf area basis, of the successive leaves from the four potash series.

four series are plotted against the amount of potash applied in the nutrient solutions. The maximum respiration rate in Series A, B, and C was attained at the fourth leaf, while in D it was reached at the third; after this maximum, B and C showed a gradual decline in rate, but in A and D a more sudden drop to a considerably lower value occurred, followed in D by a secondary rise in the later formed leaves. Manurial differences are very pronounced after about the fourth leaf; on a dry weight basis (Fig. 1) B and C are not very different, but are considerably higher than the fully manured series. At very low potash concentrations respiration rate is again lowered, so that D takes an intermediate place between B and C on the one hand, and A on the other. On a leaf area basis the percentage differences due to manuring are very much increased, and in the middle period of growth the four curves become widely spaced, the order being B (highest),



C, A, and D. These differences are considerably diminished in the last two leaves.

TABLE I.  
*Rates of Respiration.*

Dry weight basis gives mgms. CO<sub>2</sub> per hour per grm. dry weight.  
Leaf area " " " " " " " " per dm<sup>2</sup>. leaf surface.

	A.		B.		C.		D.	
Leaf No.	Dry Weight basis.	Leaf Area basis.	Dry Weight basis.	Leaf Area basis.	Dry Weight basis.	Leaf Area basis.	Dry Weight basis.	Leaf Area basis.
1	5.62	1.585	—	—	6.60	2.008	—	—
2	6.29	2.185	9.33	3.402	7.28	2.471	7.98	2.183
3	8.07	2.426	7.52	2.165	8.50	2.507	13.66	3.869
4	10.72	4.092	12.23	4.547	10.93	4.477	8.87	2.541
5	6.15	2.540	8.16	3.998	9.11	3.517	8.31	2.658
6	6.21	2.115	9.46	3.793	8.22	3.043	5.53	1.240
8	5.10	2.033	10.90	4.039	9.08	2.820	6.68	1.398
9	5.94	2.447	8.90	4.025	9.25	3.169	6.93	1.415
9	7.08	3.057	7.44	2.611	7.58	2.495	4.89	1.007
10	4.81	2.053	8.06	3.449	8.17	3.088	9.52	2.360
10	6.46	2.712	7.92	3.103	8.17	2.883	8.51	2.015
10	5.79	2.128	6.26	2.574	8.31	2.714	7.09	1.712

As the amount of potash supplied to the plant is decreased, so the respiration rate of the leaves, however it is expressed, increases until a maximum value is reached, after which further decrease in potash supply is accompanied by decrease in respiration rate.

The figures obtained have been subjected to the Analysis of Variance, the results of which are given below :

TABLE II.  
*Dry Weight Basis.*

Variance due to—	Degrees of Freedom.	Sum of Squares.	Mean Square.	<i>z.</i>	5 %.	1 %.
Manuring	3	31.53804	10.51268	0.767	0.536	0.753
Leaf Number	10	51.58761	5.158761	0.411	0.389	0.550
Remainder	30	68.01263	2.2670877			
Total	43	151.13828				

*Leaf Area Basis.*

Variance due to—	Degrees of Freedom.	Sum of Squares.	Mean Square.	<i>z.</i>	5 %.	1 %.
Manuring	3	11.992200	3.997400	1.194	0.536	0.753
Leaf number	10	8.324634	0.8324634	0.409	0.389	0.550
Remainder	30	11.018467	0.36728223			
Total	43	31.335301				

Thus on either basis there is a real effect due to treatment, as well as a real effect due to age, or leaf number. The differences between the

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means of the four treatments are given below, together with the appropriate standard error :

Comparison	Differences of means.	
	Dry Weight basis.	Leaf Area basis.
A-B	-2.142	-0.902
A-C	-1.998	-0.491
A-D	-1.396	+0.490
B-C	+0.144	+0.411
B-D	+0.746	+1.392
C-D	+0.603	+0.981
Standard error	0.642	0.258

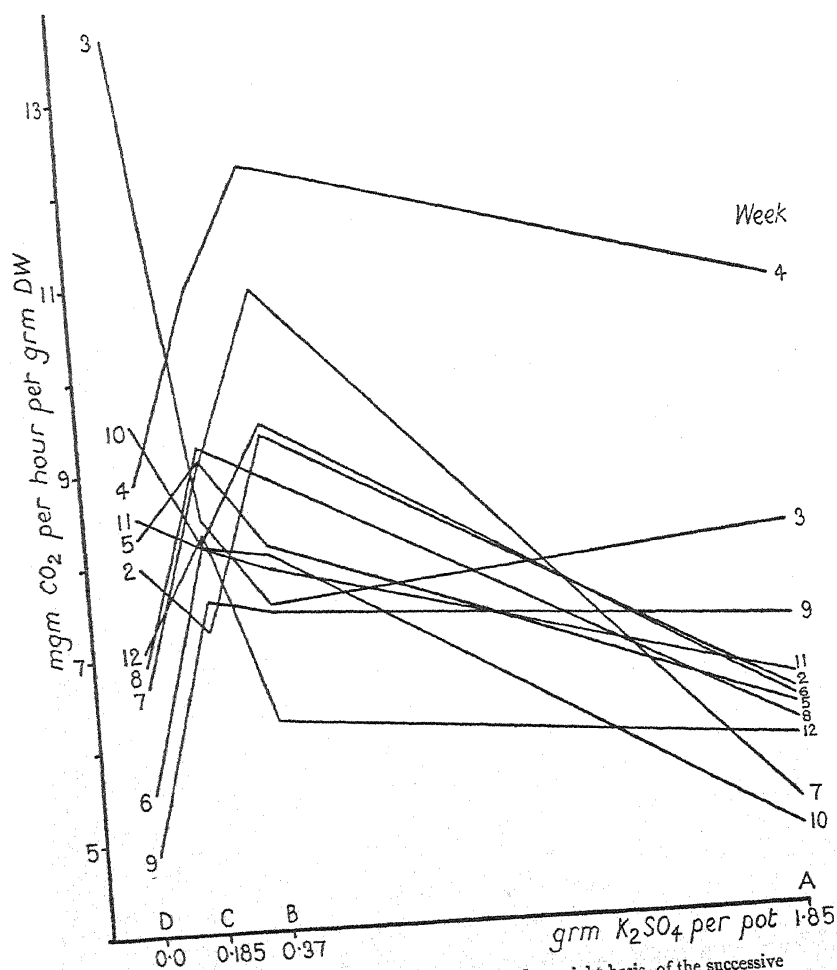


FIG. 3. Graph showing rates of respiration, on a dry weight basis, of the successive leaves, plotted against the amount of potash applied.

The differences greater than twice their standard error, those with a probability of significance greater than twenty to one, are shown in heavy type. On a dry weight basis all that is definitely proven is that A has a lower

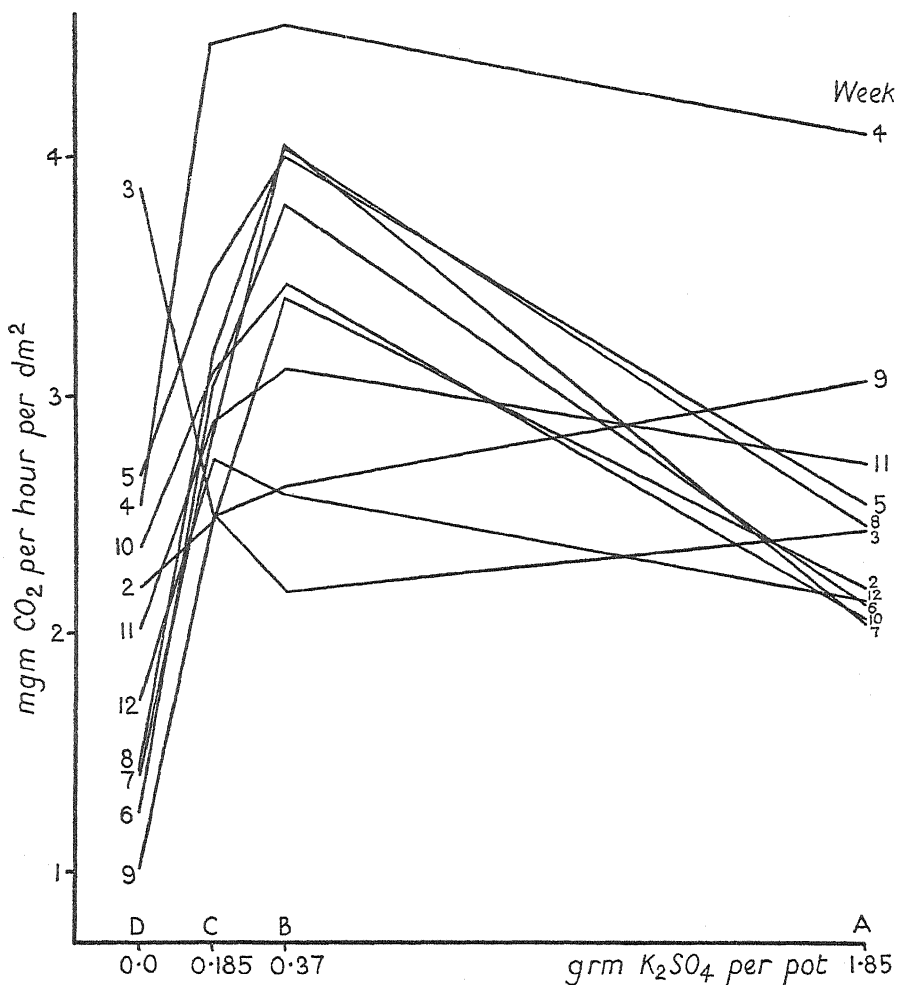


FIG. 4. Graph showing rates of respiration, on a leaf area basis, of the successive leaves, plotted against the amount of potash applied.

respiration rate than any of the deficient series, while on a leaf area basis the rate in D is shown to be lower than that in B and C, and that in A also to be lower than that in B. It is desirable also to demonstrate that on a dry weight basis the rate at very low potash concentrations (D) is lower than that at medium concentrations (B and C). A glance at Fig. 1 abundantly demonstrates that the interaction between potash concentration and leaf

number must be very considerable, the four curves being far from parallel and similarly situated; and all the variance due to this cause has been included in experimental error, which must therefore be an exaggerated estimate. It is also clear that the four values obtained from the third leaf must contribute an excessive amount to this estimate, seeing that the value for D is there much higher than are those of the other three series. It is unlikely that this is due to chance causes, but in all probability a real interaction is indicated. If the respiration rate rises as potash deficiency becomes more and more acute, Series D may be expected to show the effects of deficiency earlier than the others, and hence it seems very probable that the high value in the third leaf is real, and should not contribute an excessive amount to the estimate of experimental error. Better justification for this omission will appear later (p. 385).

An Analysis of Variance on the respiration rates (dry weight basis), omitting the four values at the third leaf, then, gives the following results:

TABLE III.  
*Dry Weight Basis.*  
(Omitting Third Leaf.)

Variance due to—	Degrees of Freedom.	Sum of Squares.	Mean Square.	<i>z</i> .	5 %.	1 %.
Manuring	3	37.31081	12.43694	1.090	0.543	0.763
Leaf number	9	42.31339	4.701488	0.603	0.408	0.577
Remainder	27	37.98459	1.406836			
Total	39	117.60879				

It is seen that by this omission the mean square ascribed to error is considerably lowered, and the significance of the *z* values much increased; the standard error shows also that, except in the earliest and last leaves, respiration rate is significantly lower in Series D than in either B or C:

Comparison.	Differences of Means.	Standard Error.
A-B	-2.411	± 0.530
A-C	-2.155	
A-D	-0.976	
B-C	+0.256	
B-D	+1.435	
C-D	+1.179	

Another apparent effect of potash starvation—a lowering of the temperature coefficient of respiration—may conveniently be mentioned here, though is not desired to press a result derived from such few data. The potash deficient series of 1927 corresponded very nearly in manuring to Series C of 1928; the mean temperature used in the former year was 17° C., and in

the latter 24°. Assuming the differences in the mean rates of respiration in corresponding manurial series between the two years to be due entirely to temperature, the value of  $Q_{10}$  in the fully manured plants is 3.02, while that in the potash deficient series is 2.43. Two actual estimations of the temperature coefficient in Series A and D were made over the range corresponding to the two years' experiments, using the sixth and the eighth leaf respectively. For the sixth leaf, the value of  $Q_{10}$  in A was 2.20, and in D 1.56; while for the eighth that of A was 1.57, and of D 1.235.

*Water Relationships of Leaves, and their Bearing on the Choice of a Basis for the Expression of Respiration Rate.*

In Table IV are given, for the four series of leaves, the values obtained for water content expressed as a percentage of the dry weight, and for the ratio of dry weight to leaf area.<sup>1</sup>

TABLE IV.

Leaf No.	A.		B.	
	Dry Weight *. Leaf Area	H <sub>2</sub> O × 100. Dry Weight	Dry Weight *. Leaf Area	H <sub>2</sub> O × 100. Dry Weight
1	30.4	577	—	—
2	36.0	570	35.9	586
3	31.0	657	31.1	741
4	40.8	573	38.5	636
5	41.3	498	49.0	476
6	34.1	577	40.1	558
8	39.9	516	37.0	543
9	41.2	502	45.2	475
9	43.2	357	35.1	411
10	42.6	236	42.8	217
10	42.0	248	39.2	258
10	36.7	279	41.1	252

Leaf No.	C.		D.	
	Dry Weight *. Leaf Area	H <sub>2</sub> O × 100. Dry Weight	Dry Weight *. Leaf Area	H <sub>2</sub> O × 100. Dry Weight
1	31.4	585	21.6	841
2	33.4	604	29.2	624
3	32.7	670	31.8	716
4	43.6	553	29.9	827
5	38.6	608	32.0	782
6	37.0	598	22.4	1053
8	31.1	741	20.9	1119
9	34.3	448	20.4	1078
9	32.9	371	20.6	783
10	37.8	290	24.8	479
10	35.3	277	23.7	457
10	32.7	307	24.1	551

\* Weight in cgms. per dm<sup>2</sup>. Leaf Surface.

If these figures are examined by means of the Analysis of Variance,

<sup>1</sup> The legend of Table I in the previous paper (5) should have read *centigrams* per sq. dm., not *mgms.*

it is found that the manurial differences in both characteristics are very much too great to be accounted for by chance; in water content, the value of  $s$  is 1.586 (1 per cent. = 0.753), while in the ratio of dry weight to leaf area it is 1.720 (1 per cent. = 0.753). If the mean values of the series are examined among themselves by means of the appropriate standard error the following results are obtained:

TABLE V.

Water Content.	Differences of Means.
D-C	+ 272.9 $\pm$ 43.2
C-B	+ 28.5 $\pm$ 43.2
B-A	+ 12.7 $\pm$ 43.2
Dry Weight: Leaf Area.	Differences of Means.
D-C	- 9.96 $\pm$ 1.65
C-B	- 4.15 $\pm$ 1.65
B-A	+ 0.56 $\pm$ 1.65

It will be seen that in water content A, B, and C have not been shown to differ significantly from one another, but that D is very significantly higher than the others; in the ratio of dry weight to leaf area A and B show no difference, but C is significantly below them, and D again very significantly below C. There can be no doubt that the difference in water content between C and either A or B is real, but that this reality is hidden by the use of a much exaggerated estimate of experimental error. As can be seen from the Table, or from Fig. 5, there is a very different behaviour in time between D and the other series and, owing to the fact that replicated values were not obtained, the variance due to this has been included in that ascribed to experimental error. This leads to a standard deviation as high as 18.5 per cent. of the mean value in this characteristic, while in the ratio of dry weight to leaf area the corresponding standard deviation is only 11.1 per cent. of the general mean.

In the previous publication it was pointed out that a high negative correlation exists in time between (1) the ratio of the water content in one manurial series to that in another, and (2) the ratio between the dry weights of unit leaf area in the same two manurial series. A diagram was there given of this interrelationship in nitrate deficient and fully manured plants, and a similar diagram is presented here (Fig. 6) for the potash Series D and A, except that the logs of the ratio values are plotted instead of the values themselves, in order to bring out the correlation more clearly. The correlation coefficient between the actual values is -0.918 (1 per cent. = 0.708). The middle curve is obtained by adding together corresponding points on the other two, and represents the log of the ratio of the weight of water per unit leaf area in D to the weight of water per unit leaf area in A. As before, this ratio is found to be much more constant than the

corresponding ratio of water content on a dry weight basis, but yet to deviate definitely if slightly from the zero line in the same direction. In Fig. 5 the weight of water per unit leaf area in these two series is shown, together

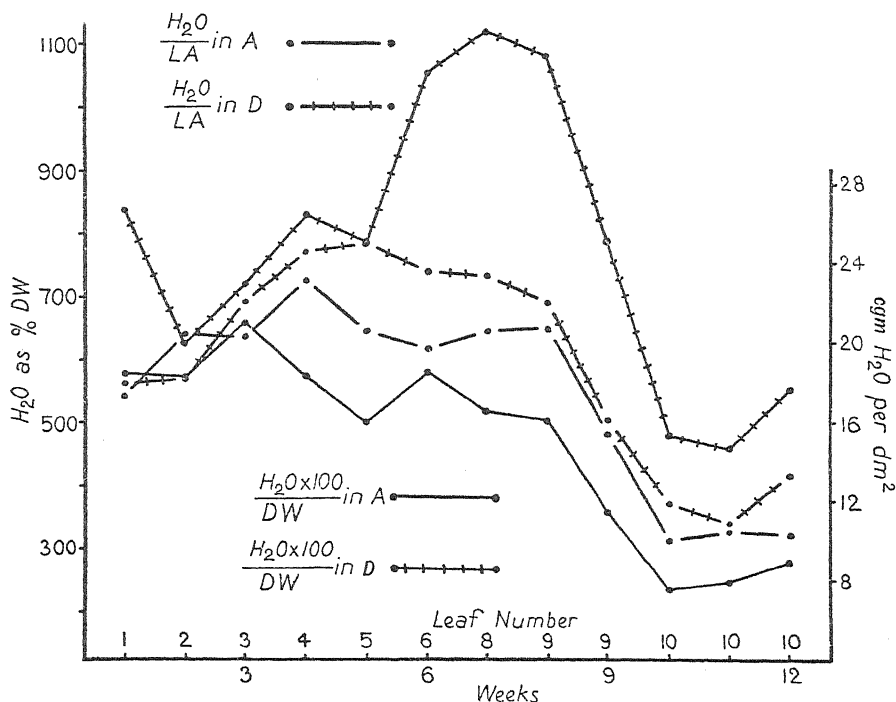


FIG. 5. Graph showing water content of the successive leaves in Series A and D, expressed in terms of both dry weight and leaf area.

with the water content expressed as a percentage of dry weight, for comparison.

The Analysis of Variance of this characteristic shows that, in spite of a considerably lower experimental error than in the ratio of dry weight to leaf area even (below 8.0 per cent. of the mean), the differences between the manurial treatments are so small that D is the only series differing significantly from the others; the difference between D and A is not so great as three times its standard error:

Water : Leaf Area.	Differences of Means.
D-C	+1845 ± 676.4
C-B	-689 ± 676.4
B-A	+811 ± 676.4

It appears, then, that under a type of mineral salt deficiency that leads to a divergence from the normal in the ratio of water to dry weight, the amount of water per unit leaf area shows a corresponding and real, though

much less pronounced, deviation. It seems fairly clear that the differences observed in the ratio of dry weight to leaf area between manurial treatments can be largely accounted for by a contraction or expansion of leaf surface due to an abnormally small or great amount of water respectively. What is not so clear at first sight is that, since real and small differences do exist in the amount of water per unit area of leaves from plants treated differently, it is quite possible that practically all the differences found between treatments in the ratio of dry weight to leaf area may be attributable to this cause.

Thoday (12), working with *Helianthus*, was the first to show that leaves will expand and contract with gain or loss of water, and some unpublished data of the present author show that this is perfectly true of leaves of barley, both under conditions of complete nutrition, and of deficiency in potash. There is no reason to suppose that leaves from barley deficient in nitrate or phosphate behave differently.

There is also little reason to expect that an expansion of leaf surface, due to an increase in water content, would not be reflected in a similar expansion in leaf thickness, and indeed, Bachmann (1) has recorded considerable changes in this dimension due to water variation. If such an expansion affects all three dimensions of the leaf proportionally, and the expanded area be  $x$  times the unexpanded, then the expanded leaf volume will be  $x^{1.5}$  times the unexpanded. If a similar relation<sup>1</sup> can be shown to hold between the various manurial types, it may then be assumed that dry weight per unit leaf area, when measured at constant water content, is unaffected by manurial treatment; since it appears unlikely that the relative expansion in the three dimensions, due to water, will be appreciably affected by the manurial treatment. For the leaves under consideration, those of the four manurial types of 1927 discussed in the previous paper, and the potash deficient series of 1928, the leaf surface representing a given dry weight is known; the leaf volume representing the same dry weight is more difficult to estimate, but if we neglect intercellular spaces, the actual weight of water associated with that dry weight will be approximately proportional to the required volume of the leaf. Another approximation will be given by the ratio of fresh weight to dry weight, seeing that the density of the living leaf substance cannot differ markedly from unity. Since a considerable portion of the dry weight must exist in the living leaf in solution, it would seem that the best estimate of volume per unit dry weight, from the data available, is one intermediate between the estimate based on water alone and that based on fresh weight.

The leaf data from the two years' experiments have been examined from this point of view. The various comparisons indicate that in general

$$\frac{\text{Vol. of unit dry wt. in treatment } x}{\text{ " " " " } y} = \left( \frac{\text{Area of unit dry wt. in } x}{\text{ " " } y} \right)^{1.5}.$$



the value of the exponent is greater than unity, and therefore that there is a real expansion in thickness accompanying an expansion in area. Values both above and below 1.5 have been obtained. It appears, then, that if the

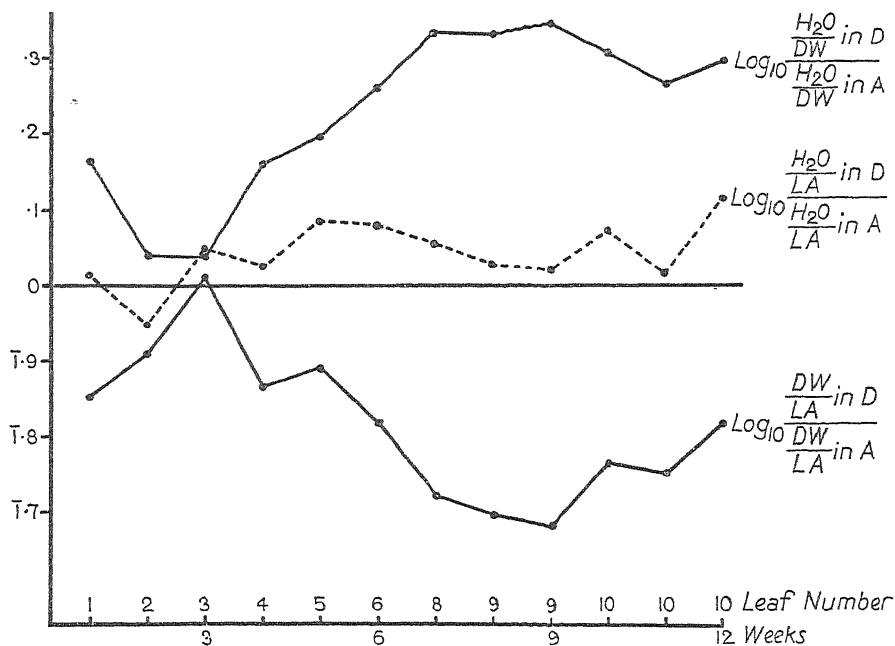


FIG. 6. Graph showing various water relationships in the leaves of Series A and D. For explanation see text.

ratio of water to leaf volume were plotted for the different manurial treatments, the curves would be still more nearly coincident than are those of the ratio of water to leaf area; and hence that, if a complete correction were applied to the ratio of dry weight to leaf area for water content, the differences between the manurial curves would become very markedly less, and almost, in fact, disappear.

Differences between the successive leaves of one plant as they emerge cannot be accounted for in the same way. Water content shows a considerable and progressive change from leaf to leaf, whereas dry weight to leaf area fluctuates more nearly about a mean value, indicating that could leaf area be measured at a uniform water content in the successive leaves, the ratio of dry weight to area would show a marked fall in the later leaves, as against the earlier. The conclusion is foreshadowed by the fact that when water content is expressed in the form of the ratio of the weight of water to leaf area the percentage difference between treatments is much diminished, while that between the successive leaves is almost unchanged.

From these considerations, the conclusion emerges that deviations in

the ratio of dry weight to leaf area due to manurial treatment are mainly of a different nature from corresponding deviations between the successive leaves of the plant. The former have very little real existence apart from water differences, whereas the latter must represent differences of leaf structure.

The preceding discussion establishes that almost all the divergence in the ratio of dry weight to leaf area brought about by the manurial differences studied is due to the extent to which the leaves are inflated with water. Hence the large differences between the curves of respiration of successive leaves, as expressed on a dry weight (Fig. 2) and a leaf area (Fig. 4) basis, are attributable to differences in water content resulting from manurial treatment. Since it is desirable to exclude from the respiration relations the action of a second variable factor affected by manuring, namely water content, it would seem preferable to express respiration results for comparative purposes in terms of dry weight. A and B show no real difference in water content; similarly the general relationship between curves A and B is almost identical in Figs. 1 and 2; on the dry weight basis, the mean respiration rate of B is 132.4 per cent. of that of A, and on a leaf area basis 135.9 per cent. C has a higher water content, and the leaf expansion in this series leads to a drop in the respiration rate on a leaf area basis to a position intermediate between A and B; while the very high water content of D leads similarly to a considerable relative drop, bringing it well below that of the fully manured series. Thus, if we consider the value of the mean respiration rate in C in terms of that in A, the ratio of this value on the dry weight basis to that on the leaf area basis is 1.0906; the corresponding ratio of the water contents in terms of dry weight, in C and A, is 1.0905. Similarly, D and A give the corresponding values 1.503 and 1.689.

#### *Potash Content of Leaves.*

The leaves used in these experiments were subsequently analysed for potash content,<sup>1</sup> and the results are presented in Table VI. The differences in potash content between leaves from Series A and those from the other series is very marked; B, C, and D are much more nearly alike in the amounts they contain. Thus, if we consider the percentage of  $K_2O$  in the dry weight of the leaves, the mean values between determinations 2 and 12 for the Series A, B, C, and D, are 2.347, 0.759, 0.671, and 0.539 respectively; even neglecting the earlier leaves, and considering the last four determinations after the minimum in potash concentration had been reached, the corresponding values are 2.718, 0.469, 0.478, and 0.341. The values obtained for potash concentration within the leaf indicate very strongly that this is much higher in the earliest and latest formed leaves

<sup>1</sup> The author again wishes to thank Dr. Janet W. Brown for undertaking these analyses.

than in the intermediate ones, but in the case of Series A the curve shows some marked irregularities. These irregularities appear to correspond largely with periods of heavy rainfall, and the correlation with rainfall has therefore been examined. In order to do this, a parabola was fitted to the curve of potash concentration in the successive leaves, and the deviations from this parabola correlated with deviations from the parabola similarly fitted to the rainfall curve, the rainfall chosen being the amount which fell during the week preceding the day on which the leaves were cut from the plant. This method is equivalent to correlating potash concentration with rainfall, eliminating from the total correlation that which may be due to the first and second powers of time, i.e. the general shape of the potash concentration curve. The coefficient obtained is negative and high,  $-0.745$ , a value which, considering the appropriate number of degrees of freedom, has a probability of significance of approximately 50 : 1.

TABLE VI.  
Per cent.  $K_2O$  on Dry Weight Basis.

Leaf No.	A.	B.	C.	D.
1	2.47	—	1.278	1.658
2	3.53	2.264	1.449	1.396
3	2.97	1.153	1.143	0.950
4	1.61	0.968	0.888	0.425
5	1.66	0.538	0.473	0.882
6	1.71	0.821	0.509	0.246
8	1.96	0.374	0.359	0.478
9	1.51	0.353	0.649	0.186
9	2.21	0.656	0.761	0.340
10	3.68	0.587	0.576	0.412
10	1.74	0.389	0.370	0.534
10	3.24	0.242	0.204	0.078

The values for rainfall used in this correlation may appear somewhat arbitrary, but this particular measure was not chosen because it gave a higher correlation coefficient than others. An ideal estimate of 'rainfall' would probably be a weighted one in which recent rain was given a higher value than rain which had fallen some days previously, from the effects of which the leaf has presumably had time partially to recover; and in fact, had such an estimate been used in the present instance, a considerably higher correlation would have been obtained.

The data from the fully manured series of 1927, when analysed in a like manner, also give a high and negative correlation coefficient,  $-0.565$ . This has a probability of significance greater than 10 : 1 but less than 20 : 1, and, though this is not usually regarded as 'significant', it points in the same direction as the 1928 data. It is interesting to note that the mean rainfall value in 1927 (0.934 in.) was much *higher*, and the rain fell more

uniformly, than in 1928 (0.544 in.), while the potash content in 1927 (mean = 6.92 mgms. per sq. dm.) was considerably and consistently *lower* than in 1928 (8.91 mgms.), which is consistent with the relation indicated by the above correlations.

Series B, C, and D do not show a similar variation with rainfall.

Mann and Wallace (8) have shown that, in the case of apple trees, potash may be washed out of living leaves by means of running water; so easily, in fact, is this done that a similar leaching occurs in the field during rainy periods. Other workers have reported somewhat similar results. The above data taken collectively indicate strongly that the same phenomenon occurs in nature in the case of leaves of barley. This is true of plants which have been given an ample supply of potash, but where this element is deficient and the internal concentration low, the absence of any correlation with rainfall would appear to show that the potash present is less readily removed and has not the comparatively loose association with the tissues which seems characteristic of much of the potash in plants having a high internal concentration.

#### GENERAL DISCUSSION.

As was found for the fully manured and potash deficient plants in 1927, there is again no demonstrable relationship in any of the four present series between the potash concentration and the respiration rates of the successive leaves with the exception of Series D. The interrelationship of these two variables between the four series also is apparently not of a simple nature, seeing that potash content drops greatly from A to B, and very slightly from B to D, whereas respiration rises from A to B, and falls markedly from C to D.

An explanation of these results may be sought in the amount of carbohydrate to be found in the leaves of plants given different amounts of potash, but, as has been shown (5), over a period in the history of partially deficient plants when the carbon assimilation rate is markedly subnormal the respiration rate of the same leaves is markedly supernormal. Furthermore, unpublished work by F. G. Gregory indicates that under moderate deficiency the leaves of barley do not have a higher sugar concentration than normal, but the reverse, and these results are supported by other workers (7, 10). On the other hand, it appears probable that the lowering of respiration rate between Series C and D is due to the low concentration of carbohydrate material, seeing that the assimilation rate (Fig. 7) in Series D was very subnormal throughout almost the whole life-history of the plants. In this connexion, the correlation coefficients between assimilation rate and respiration rate in the four series are interesting. Below are given the total correlations, and also the correlations after eliminating the general effect of time :

Series.	Total Correlation.	Partial Correlation.
A	+0.0426	-0.166
B	+0.1959	-0.011
C	+0.2356	+0.164
D	+0.8724	+0.827
1 % point	0.735	0.765

It is seen that these correlations are quite insignificant in manurings A, B, and C, but highly significant in the case of the totally starved plants, in which the curve of respiration rate reproduces closely that of assimilation rate (Figs. 2 and 7). The small positive partial correlation in Series C may be an indication that also at this level of potash manuring respiration rate is partially dependent on the assimilatory capacity of the leaves, and presumably, therefore, on the carbohydrate concentration, since the respiration rate was observed shortly after a period of assimilation.

The closeness of the agreement between respiration and assimilation rates in Series D, both being measured in terms of leaf area, is worth examining more closely. At this level of manuring, and at this level only, a high and significant positive correlation is found between respiration rate and potash content for the successive leaves. The coefficients (on a leaf area basis) are as follows:

Series.	Correlation.
A	-0.3901
B	-0.2169
C	-0.0858
D	+0.6467

} 5 % = 0.602

It was shown in the previous publication (5, p. 153) that a similar high correlation in potash-deficient plants was found to be spurious, and to be entirely dependent on the dry weight per unit area of the leaves. Here also the relationship is a spurious one, as may be seen by eliminating from the total correlation that part which is due to assimilation rate. The results are illuminating. In Table VII are presented for the four manurial series the partial correlation coefficients between each pair of the three variates, respiration rate ( $r$ ), assimilation rate ( $a$ ), and internal potash concentration ( $k$ ), all being measured in terms of leaf-area. From the total correlation between each pair has been eliminated that due to the third variate:

TABLE VII.

Series	$r_{ar.k}$	$r_{ak.r}$	$r_{rk.a}$
A	-0.325	-0.163	-0.389
B	+0.199	+0.462	-0.067
C	+0.317	+0.868	-0.234
D	+0.779	+0.655	-0.203

5 % = 0.632                      1 % = 0.765

From column 2 it is clear that assimilation rate is independent of potash concentration when this is high (A); but that the partial coefficient rapidly rises with the degree of starvation, being highly significant in C

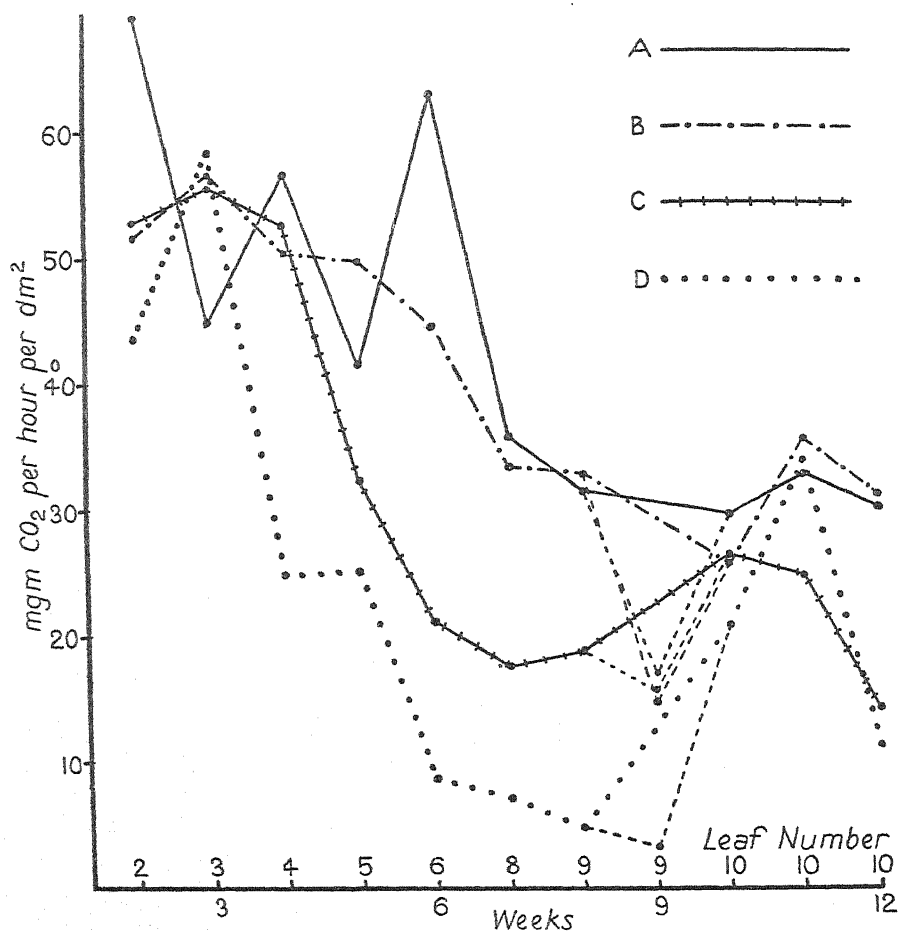


FIG. 7. Graph showing rates of assimilation, on a leaf area basis, of the successive leaves from the four potash series. Temperature, 24°C.; light intensity, 15,250 metre candles excess CO<sub>2</sub>.

and D. Column 1 shows with increasing deficiency a similar though more gradual rise in the coefficient between assimilation and respiration rates; while column 3 shows that the high positive total correlation between respiration rate and potash content in D has entirely disappeared, and that at no level of potash manuring is there any appreciable correlation between the two; what correlation there is appears to be negative. The conclusion is, therefore, that under extreme starvation (D) assimilation rate is dependent on the potash content of the leaves, and that respiration rate is in its

turn dependent on the assimilation rate. A similar condition, though less marked, is probably found in Series C; but where the potash supply is ample (A) no appreciable correlation exists between any two of the three variates.

It is not intended to discuss here the relationship between assimilation and potash except in its bearing on respiration rate. At the beginning and at the end of the life-history only does assimilation rate reach an intensity comparable with that in the fully manured series. Over the rest of the range the remarkably low assimilatory capacity is reflected in what is a subnormal respiration rate for this series (it should be remembered that the respiration rates were observed *after* a period of assimilation, so that carbohydrate concentration during the respiration period must have been highly correlated with the assimilation rates observed). It follows, then, that only in the first few and the last leaves in this series is there any measure of the intensity of respiration under conditions of adequate supply of carbohydrate. It is highly significant that these particular leaves from Series D have *higher* respiration rates than those from the other three series, when the rates are expressed in terms of dry weight, the only really comparable basis. In fact, the two first determinations on the last leaf, where, judging from the assimilation rate curves, the internal concentrations of carbohydrate must be comparable in all the series, increasing potash starvation is reflected in a continually rising respiration rate from A to D (see Fig. 3, curves 10 and 11). The rise from A to B is great, and that from B through C to D more gradual; since the internal concentration of potash within the leaves shows a sharp drop from A to B, and a further small decrease from B to D, there is some indication that so long as carbohydrate content is not in the minimum, the rate of respiration between treatments (as distinct from successive leaves from the same treatment) is negatively correlated over the whole range with potash concentration.

These considerations indicate that the very high rate observed in Series D at the third leaf is indeed a real one, and that there is a high interaction between treatment and time due largely to the different behaviour of this particular leaf; there is thus every justification for omitting these four values from the Analysis on p. 374.

The immediate cause of this potential continual increase of respiration rate with increasing potash starvation remains obscure. Under conditions of low potassium supply and ample nitrogen a high percentage of nitrogen in the plant may be anticipated, leading possibly to a larger percentage of protoplasm in the potassium deficient leaves than in the leaves of fully manured plants. Work now in progress in this Institute has shown that higher percentages of nitrogen under conditions of potassium deficiency may be obtained. Furthermore, Morse (9) apparently found increases in the percentage of total nitrogen of the vegetative parts of soy beans as a result

of potassium deficiency. Nightingale, Schermerhorn, and Robbins (10) also report slight increases in tomato. Whether from these observations, however, it may be concluded that the percentage of protoplasm has been increased seems doubtful. The latter workers rightly discriminate between storage proteins and active proteins of the protoplasm.

They further comment on the high amide and amino nitrogen content of the potassium deficient plants as compared with the fully manured controls. Similar results were found by Burrell (2) in soy beans, and have been confirmed for barley by the author in work now in progress. In this connexion the observations of Spoehr and McGee (11) on the accelerating effect of amino acids on the respiration rate of leaves is highly suggestive; and as a provisional hypothesis the increased respiration of leaves of potassium deficient plants may be attributed to the increase in amino acid content.

One further suggestion may be made in relation to the respiratory effect. Evidence has accumulated showing that potash concentration has a great influence on the production of at least some enzymes. Thus Doby (3, 4) found that in sugar beet, potato, rye, &c., amylase and saccharase are present in much greater quantities in leaves from potash-starved plants than in those from completely nourished ones. Whether 'respiratory enzymes' also are affected is uncertain, and the point need not be laboured at present, though the possibility is worth bearing in mind.

The water content data presented, and those previously published (5) indicate that under the conditions described leaves of barley are more succulent under potassium deficiency than in its presence. Our knowledge of the relationship between succulence and potassium supply is in an uncertain position, and some workers have apparently obtained results diametrically opposite to those here presented. Thus, Janssen and Bartholomew (6, 7), working with a variety of plants in sand, water, and plot culture, obtained consistently reduced water contents under reduced potassium, supply, and state that their data 'show quite conclusively that high potassium plants are more succulent than low-potassium plants'. The divergence between the two sets of results can scarcely be due to the differences in the plants used, since they included oats and Sudan grass; and the author has obtained increased succulence under reduced potassium, not only with barley, but also consistently with leaves and stems of three species of grasses, i.e. Italian rye-grass, cocksfoot, and rough-stalked meadow grass. The nutrient solutions used were, however, very different; those of Janssen and Bartholomew were entirely free from sodium, and the potassium was applied as chloride; moreover, in their case the concentration of salts at the roots was maintained approximately constant throughout the period of their experiments. It may be mentioned that Nightingale, Schermerhorn, and Robbins (10) working with tomatoes in a very similar



manner to Janssen and Bartholomew, except that potassium was replaced by sodium where the former was omitted, obtained decreased succulence under potassium deficiency in the early stages of growth, but increased succulence in the later growth.

Janssen and Bartholomew (6), and Nightingale and his co-workers (10) also report that the leaves of tomato are darker green under potassium deficiency; this again is very different from the case of the barley of the present experiments, in which, as mentioned previously (5), deficiency is characterized by light yellow-green leaves.

#### SUMMARY.

1. The part played by water content in determining the differences in the usual characteristics between leaves from barley grown under various types of mineral salt deficiency is discussed; the conclusion is reached that differences in the ratio of dry weight to leaf area between treatments are almost wholly accounted for by differences in water content, whereas the variation of this ratio from leaf to leaf on the same plant is due primarily to variation in anatomical structure.

2. Results of experiments on the respiration rate of the successive leaves from plants grown at four external potash concentrations are presented. They show clearly that, in general, as the level of potash concentration is lowered, respiration rate increases, but that there is an optimum concentration below which the rate again decreases.

3. The positive correlation between respiration rate and amount of potash supplied, at very low concentrations, is apparently entirely due to the fact that carbohydrate concentration within the leaf is in the minimum. When abundant carbohydrate is present, the evidence is that over the complete range of manuring used there is a negative correlation between respiration rate and amount of potash supplied. A theory based on the amino acid content of the leaf is put forward in explanation of this.

4. As the external concentration of potash decreases so does the internal, but the relationship is not linear. There is strong evidence that where the amount of potash within the leaves is high, much of it may be washed out by rain, but under conditions of starvation what potash there is present cannot be removed in a like manner.

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# The Effects of X-rays, Ultraviolet Light, and Heat in producing Saltants in *Chaetomium cochliodes* and other Fungi.<sup>1</sup>

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With Plates XIII and XIV and one Figure in the Text.

## I. INTRODUCTION.

VERY few experiments on the effect of X-rays on fungi appear to have been carried out. Heldmaier (4) and Leonian (5) X-rayed cultures of *Schizophyllum* and a fluctuating strain of *Fusarium* respectively; in neither case was any change in the growth-rate, variability or other effect observed. Nadson and Philippov (6) exposed young cultures of *Mucor genevensis* and *Zygorhynchus moelleri* to X-rays from a gas-tube working at a potential of 75 k.v. and 2 ma., for periods ranging from 30 to 45 minutes. They found that sexual and asexual reproduction were repressed in both species, the asexual being the less strongly affected. Saltation in *M. genevensis* was observed several times, alternating light and dark sectors being produced, the former having more sporangia and fewer zygospores than the normal strain, whereas in the latter the proportions were reversed. An orange sector was also obtained, which had few zygospores and produced orange-coloured oil globules in the hyphae. All the saltants continued true to type.

Although a relatively large amount of work has been done in subjecting different fungi to the action of ultraviolet radiation, no account has been observed of saltation being induced by this means.

Barnes (1, 2) has obtained saltation in a number of different fungi by exposing them to heat.

In the present paper several different fungi have been exposed to X-rays, and of these, three have produced saltants as a result of the

<sup>1</sup> Part of thesis approved for the Ph.D. Degree of the University of London.

treatment. One of the latter, *Chaetomium cochliodes*, Palliser,<sup>1</sup> has also saltated on being subjected to ultraviolet radiation, but has so far proved resistant to the effects of heat.

## 2. GENERAL EXPERIMENTAL PROCEDURE.

Two X-ray tubes of the Coolidge type were used; the first was a water-cooled tube, and the second a 30 ma. radiator-type. Different voltages, tube currents, and periods of exposure were employed with each tube. The effective potential used varied from 35 to 70 k.v. and the tube current from 2 to 6 ma. The periods of exposure to the rays ranged from 5 minutes to 6 hours. The water-cooled tube was only employed in the early experiments, all the experiments with *C. cochliodes* and most of those with *Phycomyces Blakesleeanus*, Burgeff (= *P. nitens*, (Agardh), Kunze) being carried out with the air-cooled tube. Cultures irradiated by means of the water-cooled tube were placed at a distance of 30 cm. from the anticathode, the rays being filtered through a celluloid screen. Both filtered and unfiltered rays from the air-cooled tube were used, the distance of the cultures from the anticathode varying from 5 to 18 cm.

Malt agar (1.5 per cent. malt, 1.5 per cent. agar) was used in all cases as the culture medium. The stock strains of the different fungi were subcultured by single hyphal-tip inocula or from colonies derived from single spores. Unless otherwise stated, each colony was allowed to grow for seven days before being irradiated. Immediately after exposure to the rays a number of subcultures were made, the inocula for which being taken from different positions on the surface of the colony. In the case of *C. cochliodes* and *Fusarium culmorum* spore suspensions were also irradiated. All cultures were incubated at 25° C.

## 3. EXPERIMENTAL DETAILS AND RESULTS.

Ten different fungi were irradiated. The experiments with *P. Blakesleeanus* and *C. cochliodes* are described in detail in section (b) and in subsequent sections. The remaining fungi, in which little or no effect was obtained, are dealt with in section (a).

### (a) *The Effect on Collybia, Trametes, &c.*

Young cultures of *Collybia velutipes*, *Trametes serialis*, and *Merulius lachrymans*, which had not completely filled the Petri dishes, were irradiated, and the colonies, which in this case were not subcultured, were left to continue their growth. Apart from a slowing up of the growth rate, which

<sup>1</sup> I have to thank Mr. J. Ramsbottom of the British Museum (Natural History) for confirming my identification of this fungus.

was almost certainly due to the effect of heating during irradiation, no change was observed.

Irradiation of *Eidamia viridescens* in most cases caused the microspores to develop more rapidly, and the number of spore groups to be increased. In some cases, however, no difference was produced.

No effect was observed on irradiating either colonies or spores of *F. culmorum*, which, however, showed slight variability in the colour of the aerial mycelium in colonies derived from irradiated cultures and also in untreated colonies. In one of the latter a saltant sector arose which did not produce any coloration of the substratum. Cultures of *F. fructigenum* were also irradiated with no result.

*Mucor hiemalis* showed no effect as a result of the treatment, but *Mucor genevensis* produced a number of saltant sectors. These were somewhat darker in colour than the parent strain, owing to the presence of greater numbers of zygospores. The number of sporangia was about the same as in the parent. No orange sector, such as Nadson and Philippov obtained, was produced.

#### (b) *The Effect on P. Blakesleeanus.*

Fifty-eight cultures (half being of the plus and half of the minus strain) of this fungus were irradiated, and from them 600 subcultures were made. The potential used was 50 k.v. at 2 ma. The distance of the fungus from the target was 8 cm., and the periods of exposure ranged from 40 minutes to 3 hours. Altogether seventeen saltant sectors were observed, and eleven obtained in pure culture. Of the latter, two arose from the plus strain as a result of 3 and 1 hours' irradiation respectively; they appeared to be identical morphologically. No sporangia were produced in either case, and on pairing with the minus strain very few zygospores were formed, though such as were produced appeared to be normal. An orange material was produced in the submerged mycelium (Pl. XIII, Fig. 1). Both saltants continued true to type six months after their first appearance.

All the remaining saltants arose from cultures irradiated for 40 minutes. In five of these the sporangia developed earlier, and were produced in greater numbers than in the parent strain. After three months' time the difference between these saltants and the parent, though still evident, was not so marked as on their first appearance. Four of the five saltants arose from the minus and one from the plus strain, and apart from the differences stated above were identical with the parent. The eighth saltant, which continued true to type, arose from the minus strain and produced a smaller number of sporangia than the parent, and also few zygospores on pairing with the plus strain. An orange-coloured substance was formed in the submerged mycelium.

The three remaining saltants had a relatively slow rate of growth and

produced no sporangia. Two arose from the plus strain, and produced an orange-coloured substance in the submerged mycelium; the third, which arose from the minus strain, appeared normal in this respect, only a very small amount of colouring matter, if any, being formed. On pairing these three saltants with the appropriate parent strains very few zygospores were formed, they developed relatively slowly, and never attained full size.

Some of the above saltants arose from colonies which, during irradiation, had been exposed to a relatively high temperature ( $40^{\circ}\text{C}.$ ) for short periods. Later, however, the X-ray tube was enclosed in a different box which allowed better ventilation, and the air temperature at the position which the cultures (from which were obtained the remaining saltants) occupied did not exceed  $25^{\circ}\text{C}.$

In order to ascertain whether saltation could be induced by temperatures comparable with those obtaining during some of the above experiments, cultures were exposed to the heat from a carbon filament lamp, their distance from the latter being so adjusted that a thermometer placed at the side of the culture recorded the same range of temperatures during similar periods of time as was recorded in the X-ray box. No saltation was, however, obtained by this means.

Since no saltants were obtained in over 100 control plates of *P. Blakeleeanus*, it seems evident that those obtained from irradiated plates were due to the action of the rays.

(c) *The Effect of X-rays on Cultures of C. cochliodes of different Ages.*

*C. cochliodes* was isolated in September 1929 from an Antirrhinum stem, and on irradiating cultures saltant sectors were found to occur frequently (Pl. XIII, Figs. 2–6). Over 100 colonies, each derived from an inoculum consisting of mycelium and spores, and 600 colonies derived from spores alone have since been grown, and in no case has a saltant sector been observed, so that it appears that under normal conditions the fungus is quite stable, or saltates very rarely.

*Saltation and Age.* In order to ascertain the effect of age upon the rate of saltation induced by X-rays the following experiment was carried out. Colonies derived from inocula of approximately equal size and taken from the growing edge of another culture were grown for seven days in Petri dishes, by which time they were about 9 cm. in diameter.

In each colony two circular cuts were made in the medium, the centre of each circle being the centre of the colony. The first circle was 1 cm. in radius, the second had a radius 1 cm. less than that of the colony. The annular portion between the two circular cuts was then removed, and the remaining portions of the colony were each divided into two equal parts. The halves of the central (oldest) piece were then separated from one another in the dish, and a sheet of celluloid was placed over the dish to keep the

cultures free from contamination during radiation. Over one-half of the dish a sheet of lead 2 mm. thick was then placed to screen from the X-radiation the pieces beneath. Thus in each dish two pieces of the colony, one old, the other young (from the outside annulus), could be exposed to the rays, and two equivalent pieces (the controls) shielded from them by the lead screen. The dish was then placed at a distance of 18 cm. from the target of the tube and irradiated for 5 hours, the voltage being 50 k.v. at 2 ma. The maximum temperature recorded by a thermometer placed in the position occupied by the fungus was 20° C. Eight colonies were irradiated, and immediately following irradiation ten subcultures were made from each of the four pieces of each colony, the inocula of the subcultures being as nearly as possible of equal size. The subcultures were allowed to grow for fourteen days when the number of saltant sectors was counted in the 300 available cultures, and the following numbers of saltants per 100 subcultures were obtained:

	Control.	Irradiated.
Young material (edge)	1.3 %	38.7 %
Old material (centre)	5.3 %	92.0 %

The young portions of the colony at the time of irradiation were composed of mycelium only, whereas in the older portions perithecia were also present. The experiment was repeated, the positions of the younger and older pieces being reversed, i.e. the younger pieces were at the centre of the dish, and the older at the periphery. In this case a total of 310 subcultures was available and the numbers of saltants per 100 subcultures were as follows:

	Control.	Irradiated.
Young material (centre)	2.7 %	32.5 %
Old material (edge)	2.7 %	94.9 %

The effect of reversing the positions of the young and old pieces of the colony only served to increase the difference in the numbers of saltants produced in the irradiated material, so that the difference in rate of saltation of the material of different ages must be due to the effects of age, or to the presence of spores and a greater amount of mycelium in the older material, and not to the relative positions of the material during irradiation. The rates of saltation of the old and young portions of the colony due to irradiation are therefore in the ratio of 29/11, or the older material saltates 2.6 times as readily as the younger.

*Effect of ionization of the air.* Since under ordinary conditions of culture no saltants have been observed, and the lead screen was sufficiently thick to stop even the hardest rays produced by the tube at the potential used, it is probable, though the results are not sufficiently numerous to provide conclusive proof, that the saltants observed in the controls were due to some indirect effect of the rays, such as ionization of the air. This conclusion is

supported by the relative numbers of saltants produced in the controls. The lead screen used continued beyond the periphery of the Petri dish for at least an inch, but it did not overlap the fungal culture in the centre of the dish by more than 0.25 in., so that the fungal material in this position was considerably nearer a source of strongly ionized air than was that at the periphery of the dish, and, having allowed for the different ages of the fungal material in the two positions, the number of saltants produced in the controls at the centre of the dish remains greater than that at the periphery.

In the above experiments the three most common types of saltant character and their frequency of occurrence in 100 subcultures from irradiated material were as follows:

A darker substratum than the parent	. . .	17
Very few or no perithecia	. . .	17
Perithecia smaller than the normal	. . .	7

Each of these three characters did not in general occur alone, but appeared in conjunction with some other saltant change. Thus, for example, a saltant producing a darker substratum and very few perithecia occurred on several occasions. Some of the different types of saltants produced in this experiment are shown in Pl. XIII, Figs. 10–12; Pl. XIV, Figs. 13–16.

(d) *The Effect of Ultraviolet Radiation on the Mycelium and Spores of C. cochliodes.*

Three colonies of *C. cochliodes* were exposed to the rays from a mercury-vapour lamp for periods of 20, 30, and 60 minutes respectively, the distance from the lamp being 26 cm. Immediately following irradiation twenty subcultures were made from each of the irradiated colonies, and were allowed to grow for fourteen days. No saltants were obtained, however, in any of the sixty subcultures.

*The effect with spores.* Under the conditions of culture employed, the ascospores were extruded from the perithecia in about three weeks from the time of inoculation, and these extruded spores were used in the preparation of a spore suspension, a few drops of which were placed in each of a number of Petri dishes containing malt-agar, and were spread over the surface of the latter by means of sterilized glass rods. These dishes were then exposed to the action of the rays from the mercury vapour-lamp for 50 minutes, and were then incubated for two days. The death-rate was very high, but 206 colonies were obtained and were subcultured on to fresh dishes, twelve inocula, each from a different colony, being placed in each dish. Incubated for fourteen days, a number of colonies were found to show saltant characters; these were in every respect similar to those obtained with X-radiation. An average of thirteen saltants were produced for each 100 colonies subcultured.



(e) *The Effect of X-Radiation on the Saltants of C. cochliodes.*

Cultures of ten different saltants were irradiated for four hours, and thirty subcultures made from each. It was found that the subcultures derived from nine of the irradiated saltants showed variant sectors, the tenth saltant, No. 21 *a*, remaining true to type.

The variant sectors differed from their parents in much the same way as the latter did from the original strain. Greater or less aerial mycelia were produced, perithecia were reduced in number, an increase or decrease in the colour of the substratum occurred, and the colour of the perithecia was altered. Characters which had changed to produce the parent saltant were in some instances again altered. For example, in a variant sector produced in the saltant No. 7 *a* the colour of the substratum changed back to that of the parent strain, and in another sector it became still darker. In the saltants No. S.S. 8 and No. 13 sectors arose in which no perithecia were produced, though both saltants have more perithecia than their parent. In No. 13 another sector showed a still further increase in the number of perithecia.

It would appear then that in so far as some characters are concerned, e.g. the colour of the substratum, a reverse change is possible, though whether this can be looked upon as a true reversal to the parent strain cannot be decided in the absence of breeding experiments.

(f) *The Characteristics of the Chaetomium Saltants.*

Altogether several hundred saltants have been observed, and of these thirty-eight have been kept in culture. Only five of the latter have shown any tendency to vary; the rest, whether produced by means of X-rays or ultraviolet light, having maintained all their characters unchanged.

There was no essential difference between the characters exhibited by saltants induced by X-raying either spores or mycelium, or between saltants arising as a result of X-ray irradiation and those induced in response to ultraviolet treatment. Also, saltants produced by short exposures to X-rays only differed from those following long exposures in their frequency of occurrence and not in the type of saltant character.

Many more colonies have been grown from X-rayed material than from that subjected to ultraviolet rays, and several saltant characters have been obtained following the former treatment which have not appeared as a result of the latter. There is, however, no reason to believe that these would not be produced by ultraviolet radiation, were larger numbers of saltants obtained by this means.

A large number of different saltant characters have been obtained; these may occur singly, or a number of them may be exhibited by the same saltant. Any one character can apparently be produced independently

of any other. Thus, for example, light-coloured hairs on the perithecia may occur alone, or associated with a greater amount of aerial mycelium, or with a reduction in the number of perithecia. Also, many of the saltant characters, such as the size of the perithecia and the intensity in the colour of the substratum, showed themselves in very different degrees.

The following descriptions are of the parent strain and of some typical saltants obtained from material treated with X-rays. Only those characters are noted in which the saltant and parent strains differed from one another.

*Parent strain.* The perithecia were evenly distributed over the surface of the colony. The bodies of the perithecia were dark brown or black, and the hairs, which were coiled spirally, were dark citrine<sup>1</sup> in colour. The colour of the substratum was amber yellow, and the aerial mycelium, which was somewhat scarce, was olive-yellow (Pl. XIII, Fig. 9).

*Saltant No. 1.* The perithecia were more plentiful than in the parent strain, the perithecial hairs being deep chrome in colour. This saltant occasionally produced sectors in which no perithecia were formed.

*Saltant No. 2.* This saltant produced no apparent coloration of the substratum. The perithecia were smaller than in the parent, contained no asci and had straight hairs. Sectors occasionally appeared which contained no perithecia, but produced a considerable quantity of white aerial mycelium (Pl. XIII, Fig. 12).

*Saltant No. 3.* This saltant was completely sterile, and produced no coloration of the substratum. The aerial mycelium was white and very abundant.

*Saltant No. 4.* A normal number of perithecia was formed of about the same size as in the parent strain, and a large number of much smaller perithecia was also produced. The larger perithecia had spirally coiled hairs and produced ascospores, the smaller were sterile and had straight hairs. All the perithecia, but especially the smaller ones, had hairs of a somewhat lighter colour than the parent.

*Saltant No. 7 a.* A strong yellow ochre coloration was produced in the medium, and there was a greater coloration of the submerged hyphae than was found in the parent strain. The perithecia at the centre of the colony occurred in scattered groups of two or three, and the coloration of the substratum round them was more marked than in other parts of the colony (Pl. XIV, Fig. 16).

*Saltant No. 13.* A dense browning was produced in the substratum. The perithecia were normal, but much more numerous than in the parent (Pl. XIII, Fig. 11).

*Saltant No. 21 a.* The aerial mycelium was more plentiful than in the parent, and white in colour. There was no coloration in the substratum.

<sup>1</sup> The colours were obtained by comparison with Ridgeway's Color Standards and Color Nomenclature.

No perithecia were produced, but numerous white clumps of hyphae were formed on the surface of the medium.

*Saltant No. 33.* This strain formed a somewhat greater amount of aerial mycelium than the parent. A few normal perithecia scattered evenly over the surface of the colony, and very many smaller perithecia were produced. The larger perithecia developed ascospores, the smaller were sterile, and the hairs on the latter were straight, whereas those of the former were coiled spirally like the parent. This strain has been subcultured from single hyphal-tip inocula on several occasions, but the resultant colonies invariably gave rise to the two types of perithecia, so that this appears to be a characteristic of the saltant, and not a result of admixture of two strains (Pl. XIV, Fig. 14).

*Saltant No. S.S. 1.* The perithecial hairs were darker, and a larger amount of water was excreted by them than was the case with the parent strain. The water collected in drops on the hairs, and gave the colony a steel-blue appearance by reflected light.

*Saltant No. S.S. 3.* The perithecial hairs were black, and did not taper as in the parent, but were short and blunt. A greater number of the perithecia extruded spores and a greater quantity of spores was extruded per perithecium than was normally the case.

*Saltant No. S.S. 8.* The perithecial body and hairs were antique-brown in colour. The number of perithecia was greater and their size less than in the case of the parent.

*Saltant No. S.S. 9.* The number of perithecia was increased, and the colour of the substratum was nopal red by transmitted light.

The saltants which under normal conditions did not show any variation saltated freely on being X-rayed. The following saltants were derived from the original saltant types by this means.

*Saltant No. 7a. 1.* This saltant was similar to 7a from which it was derived, except that the colour of the substratum was vivaceous-rufous by transmitted light, in place of the yellow ochre colour of 7a.

*Saltant No. S.S. 8a.* This was similar to its parent, S.S. 8, except that the perithecia and hairs were naphthalene yellow in colour.

Changes other than those noted above also took place. For example, the length of the perithecial hairs and their number varied, and the colour of the body of the perithecium, which is dark brown or black in the parent strain, assumed a blue-black, or in other cases a pale yellow colour. Slight differences in the growth rates of the saltants and the parent strain were also observed, but in no case was any marked divergence obtained.

(g) *The Effect of Heat on Spores of Chaetomium.*

A suspension of ascospores was prepared, and 1.5 c.c. were placed in a test-tube, and heated in a water bath at 45°C. for 2 minutes. A few

drops of the heated suspension were then placed in dishes containing malt agar and spread over the surface of the latter. The experiment was then repeated, the suspensions being heated to 55°, 67°, and 80° C. respectively. After incubating for two days the colonies were subcultured as in the case of treatment with ultraviolet radiation. Very few spores survived heating at 80° C., and no saltants were obtained in the colonies derived from these. At 67° C. the number which survived was somewhat greater, but although 249 colonies were subcultured no saltants were obtained.

(h) *The Effect of Intermittent X-radiation on the Numbers of Saltants produced in Chaetomium.*

Two equivalent strips from a thirteen-day-old culture of *C. cochliodes* were each irradiated for 4.5 hours. The first was irradiated continuously, the second was irradiated for 2.25 hours and 48 hours later for a further 2.25 hours. Forty subcultures were then made from each strip, each inoculum being placed in a separate Petri dish. After fourteen days the number of saltants in each plate was counted, and the following numbers of saltants per subculture obtained.

	Mean.		S.E.
Continuous irradiation	1.43	±	0.11
Intermittent irradiation	1.6	±	0.115

The difference of the means is only  $0.17 \pm 0.159$ , which is clearly not significant.

Thus no significant difference was found in the number of saltants induced by continuous and intermittent radiation respectively, when the total period of exposure to the rays was the same in each case.

(i) *The Effect of Alteration in Wave-Length of the X-Rays on the Type of Saltant produced.*

In order to ascertain whether an alteration in the wave-length of the X-rays produced any effect on the type of resultant saltant the following experiment was carried out. A suspension of the spores of *C. cochliodes* was prepared and divided into two equal parts, one part was irradiated at 70 k.v. and 3.5 ma., the second at 40 k.v. and 2.5 ma. Assuming that the intensity is directly proportional to the tube current and to the square of the voltage, the periods of irradiation necessary to give the same dose in each case were in the ratio of 1.0 to 4.3. The suspension subjected to the harder rays was therefore exposed for 1 hour, and that exposed to the softer was irradiated for 4.3 hours. Drops of the irradiated suspensions were spread over the surface of malt-agar medium contained in Petri

dishes, and the latter were then incubated for two days when the colonies produced by the growth of the treated spores were subcultured. This method does not allow of an exact comparison being made between the rates of saltation in the two cases, but within the limits of the method no significant difference was found in the numbers of saltants produced per hundred subcultures made. There was also no difference in the type of saltant produced by the rays of longer and shorter wave-length.

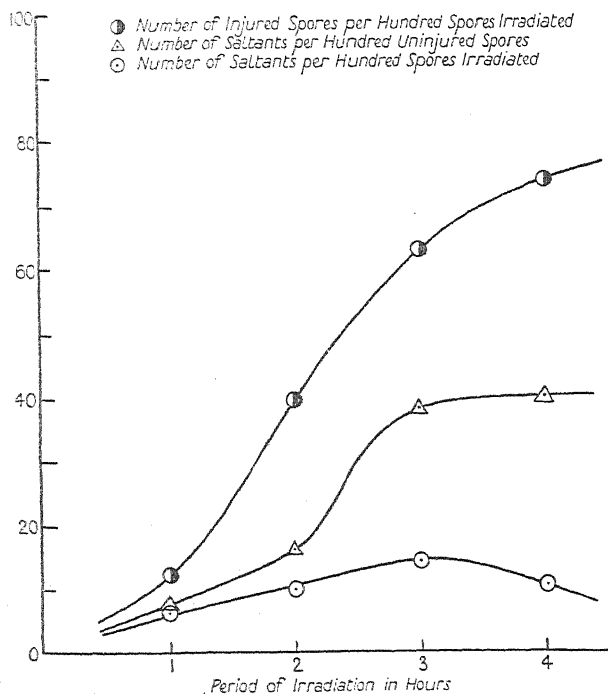
(j) *The Effect of X-Rays on the Germination and Growth of the Ascospores of Chaetomium.*

A strong suspension of ascospores was prepared and divided into two parts, one of which was irradiated for 6 hours. Drops of both the irradiated and the untreated suspensions were placed on plain agar in Petri dishes and incubated for 12 hours. On examination at the end of this period it was found that a number of spores had begun to germinate in both the treated and control material. The spores were then killed by pouring alcohol over the medium, and stained with Cotton Blue, which served to differentiate the germ-tubes from the agar-gel. The numbers of non-germinated spores in the irradiated and control material were then counted, but no significant difference was obtained, the number for the controls being 3.17 per cent.  $\pm 0.57$ , and that for the irradiated spores 2.13 per cent.  $\pm 0.63$ . It appears from this that X-radiation has little or no effect on the number of spores which germinate. It was evident, however, from other experiments that the percentage of mycelia, arising from such spores, which continued to grow until the colonies were visible to the naked eye, fell off rapidly with increasing exposures to the rays, and in order to ascertain the relative numbers of spores whose growth was limited in this way the following method was adopted. A suspension of spores was prepared and divided into two parts, one being used as the control, the other was exposed to the action of the rays. The potential used in this case was 70 k.v., and the tube current 4 ma., the distance of the suspension from the target being 18 cm. At the end of one, two, three, and four hours' irradiation respectively, portions of the suspension were removed, and spread over malt-agar in Petri dishes, and the latter incubated for 12 hours. Inocula, consisting of single germinated spores obtained from the differently treated plates were placed on fresh malt-agar, twelve inocula being placed in each Petri dish. Altogether 960 single-spore inocula, distributed among the four periods of treatment and the controls, were made. The dishes containing the inocula were incubated for seven days, when the number of colonies produced in each dish was counted, and the following numbers of spores, which had germinated, but were so injured that growth ceased after a very brief period of development, were obtained.

TABLE I.

*Number of germinated spores which were injured.*

Period of exposure in hours	1.	2.	3.	4.
Percentage of injured spores	$12.1 \pm 2.61$	$39.4 \pm 4.6$	$62.7 \pm 3.00$	$73.5 \pm 2.71$



Experiments J and K. Curves of closest fit.

The results are represented graphically in the figure above, from which it can be seen that the rate of increase in the number of injured spores takes the form of a sigmoid curve.

An inspection some 24 hours after irradiation of the plates on which the suspensions had been spread, revealed the fact that the average size of the individual colonies decreased as the period of exposure to the rays increased, and also that the degree of variation in the size of the colonies exposed for four hours was much greater than in the controls. A count of the relative numbers of small colonies in the controls and in the different periods of irradiation gave a curve reasonably comparable to that obtained in the above experiment for the number of injured spores.

From the results obtained it appears that though X-rays have only a very small effect, if any, on the relative numbers of spores which germinate, they have a marked effect in preventing the subsequent growth of the colony.

(k) *The Numbers of Saltants induced by different Periods of Exposure to X-rays.*

The method of determining the relative numbers of saltants produced as the period of exposure to the rays was increased, was the same as that used in estimating the numbers of injured spores, and the same material served for both estimations. The number of injured spores having been obtained, the dishes were reincubated for a second period of seven days, when the number of saltants for each period of irradiation was counted. The values so obtained are given in Table II. The results are represented graphically on p. 400.

TABLE II.

*Numbers of Saltants produced by different Periods of Irradiation.*

Period of irradiation in hours . . .	1.	2.	3.	4.
Number of saltants per 100 spores irradiated	6.1	9.85	14.2	10.3
Number of saltants per 100 uninjured spores	6.9	16.2	38.0	38.9

## 4. DISCUSSION.

The fact that the three fungi which have produced saltants as a result of X-ray irradiation are very stable under ordinary conditions of growth, whereas *Fusarium*, which normally shows considerable variability, has proved resistant to the rays, indicates that there is little or no correlation between the normal variability of the fungus and its capacity to produce saltants under the influence of the rays.

It is of interest, especially in view of the positive results obtained by Barnes (1, 2) in the production of saltants in various fungi by means of heat, that *Chaetomium cochliodes* is completely resistant in this respect, whereas it saltates freely on exposure to both ultraviolet light and X-rays. Since the characters of the saltants produced by both X-rays and ultraviolet light are of a similar kind, and are in some cases indistinguishable from one another, it is probable that they are produced in much the same way by the two agents.

On irradiating primary saltants, the secondary saltants produced are in some cases indistinguishable from other primary saltant strains, thus indicating that the same result can be produced in at least two stages. This fact suggests that saltation may be induced by one or more separate 'hits', such for example as the ionization of one or more specific molecules (or one or more molecules of a particular kind) in the nucleus, and that some saltants have been produced by a larger number of such hits than others. The different saltant characters are produced apparently independently of one another, so that no one variant character is always associated with any

other. In view of this it appears impossible that the only difference between one saltant and another is in the number of 'effective hits' which have been responsible for their appearance, for if this were the case and one hit reduced the number of perithecia and a second hit altered the colour of the substratum, then a saltant in which the colour of the substratum was altered would in all cases show a reduction in the number of perithecia. Therefore if it is assumed that saltation is induced by the ionization of certain molecules as suggested above, it is necessary to suppose that each saltant character is produced as a result of the ionization of one or more specific molecules (or one or more molecules of a particular kind) and that the molecules which are concerned in the production of any one variant character are different from those which take part in the production of any other such character.

The injurious effect of the rays leading to cessation of growth after a very limited period of development may also be due to specific hits, or may be produced in some other manner. In the former case injury and saltation could either be independent, as would be the case if the hitting of certain molecules was followed by injury, and that of other specific molecules by saltation, or these two could be dependent on one another in the event of the same molecules (or kinds of molecules) being affected in two cases, a larger number than is the case with saltation requiring to be hit before injury ensued. In the event of the effects occurring independently, the curve showing the number of saltants per hundred uninjured spores, i.e. those with unlimited growth, would represent the true increase in the number of saltants produced as the periods of irradiation were increased. This would no longer be the case, however, in the event of saltation and injury being dependent upon one another in the sense suggested above, and if this were so all that could be said would be that at the different doses the number of spores which had been hit sufficiently often to cause saltation, but not so frequently as to produce injury leading to a cessation of growth, was represented by the curve showing the number of saltants per hundred spores irradiated.

If the 'effective hit' hypothesis is right, and hitting takes place at random with a relatively small chance of occurring, the hits will be distributed in a Poisson Series (Fisher (3)). Assuming that the number of hits is small in comparison with the number of molecules available, the mean number of effective hits will be practically proportional to the time of exposure. It is thus possible to calculate the relative chances of a spore being hit once, twice, or more times at each period of exposure and for different values of the mean. This has been done, and the values calculated, and those obtained by experiment for the different periods of exposure are given in Table III. It can be seen that the agreement is reasonably good in each case.



TABLE III.

Mean number of hits per spore.	Number hit more than once. % calc.	Number injured. % found.
0.7	15.6	12.1
1.4	40.8	39.4
2.1	62.2	62.7
2.8	76.9	73.5

It seems probable that further light might be thrown on the matter if larger numbers of spores were grown and the frequency of occurrence of individual variant characters was noted at each of the different periods of exposure. Such estimations would, however, have to be confined to a few carefully selected characters, as many of the types of variant character which occur with any considerable degree of frequency form almost continuous series. In each of these series the saltants differ from one another only in the degree to which the variant character is expressed, such as for example different degrees of intensity in the colour of the substratum.

### 5. SUMMARY.

Ten fungi in the form of both mycelium and spores have been exposed to the action of X-rays, and of these *Mucor genevensis*, *Phycomyces Blakesleeanus*, and *Chaetomium cochliodes* have produced saltants. A description of the characters of the different saltants is given.

It has been shown that in *C. cochliodes* the old portions of a culture saltate more frequently than does the younger material under irradiation with X-rays.

The majority of the saltants obtained both by treatment with X-rays and ultraviolet light have remained true to type.

On irradiating with X-rays the saltants of *C. cochliodes* produced by that treatment, saltants were obtained which also continued true to type.

A reduction in the hardness of the X-radiation did not alter the type of saltant produced.

X-rays have little effect on the germination capacity of the ascospores of *C. cochliodes* up to a maximum dose of six hours. Irradiation has, however, a marked effect on the subsequent growth of the spores, and with increasing times of exposure up to a maximum of four hours, more and more of the spores developed only a small mycelium.

Heating the ascospores of *C. cochliodes* did not cause saltation.

In a study of the behaviour of about 900 irradiated ascospores of *C. cochliodes*, the relationship between the time of exposure to X-rays and the number of saltants produced was determined. The nature of the X-ray effect in producing saltation is considered, and the effect of separate ions

produced by the rays on particular constituents of the nucleus is put forward as a possible cause of saltation.

I wish to express my sincere thanks to Professor V. H. Blackman for his interest and helpful criticism.

I also have pleasure in thanking Mr. H. Tooley for his help in taking the photographs, and Mr. W. Shaw for his technical assistance.

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#### EXPLANATION OF PLATES XIII AND XIV.

Illustrating Dr. Hugh Dickson's paper on The Effects of X-rays, Ultraviolet Light, and Heat in producing Saltants in *Chaetomium cochliodes* and other Fungi.

##### PLATE XIII.

Fig. 1. *Phycomyces Blakesleeanus*. A subculture from an X-rayed colony showing a saltant sector in which no sporangia were produced, and an orange-coloured substance was present in the submerged mycelium.

Figs. 2-6. *Chaetomium cochliodes*. Subcultures from X-rayed colonies showing various types of saltant sector.

Fig. 2. In one of the two large sectors no perithecia are formed, while in the second they are produced in great numbers, and are smaller than in the parent, and have hairs of a brown colour.

Fig. 3. In one of the sectors no perithecia are produced, in the others the perithecia are more numerous and smaller than in the parent.

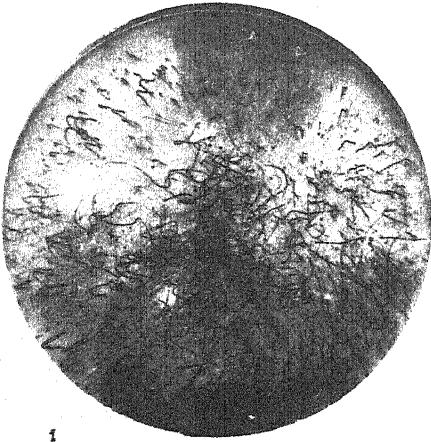
Fig. 4. A greater amount of aerial mycelium is produced in the saltant sector, and the perithecia are small and few in number.

Fig. 5. A strong coloration of the substratum is seen in the saltant sector, and no perithecia are produced.

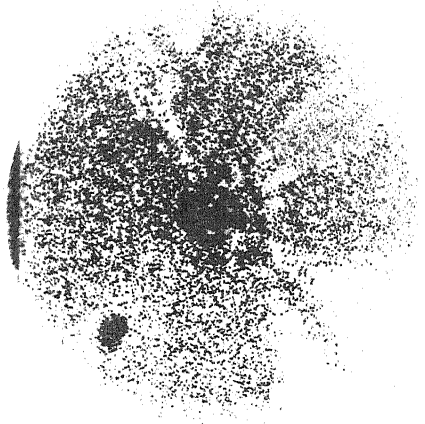
Fig. 6. The two narrow sectors have similar characters to those of the saltant in Fig. 5. The narrowness of the sectors is very typical of this type of saltant.

Figs. 7 and 8. Colonies derived from X-irradiated spores of *C. cochliodes*. Several colonies have saltated in each plate, the variant characters of which include a reduction in the number and size of the perithecia, a brown coloration of the substratum, and light-coloured perithecial hairs.

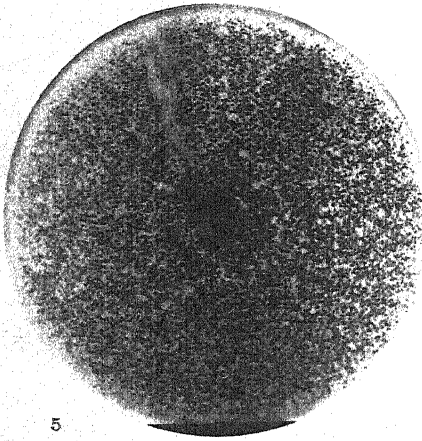




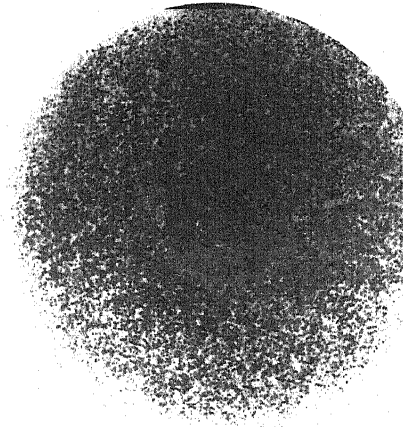
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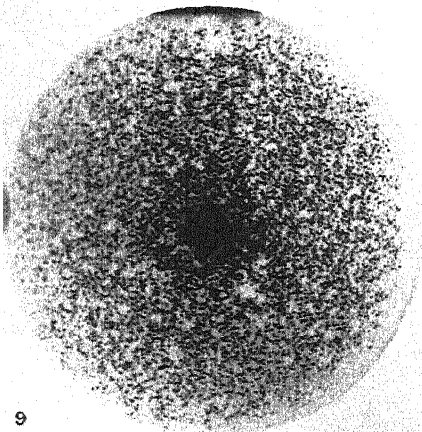
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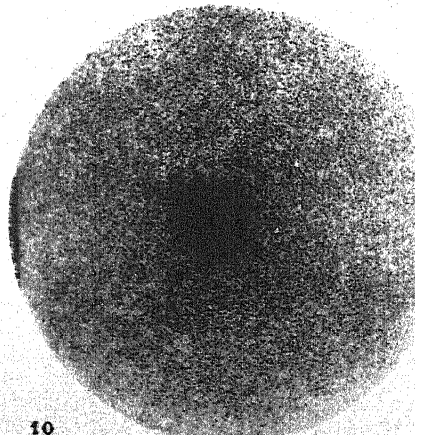
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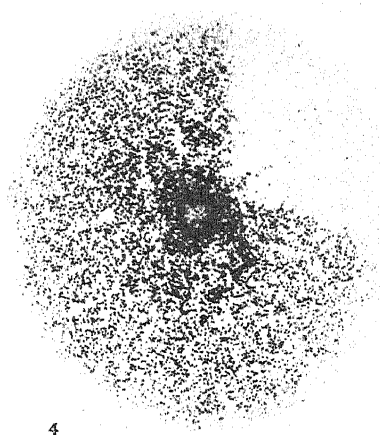
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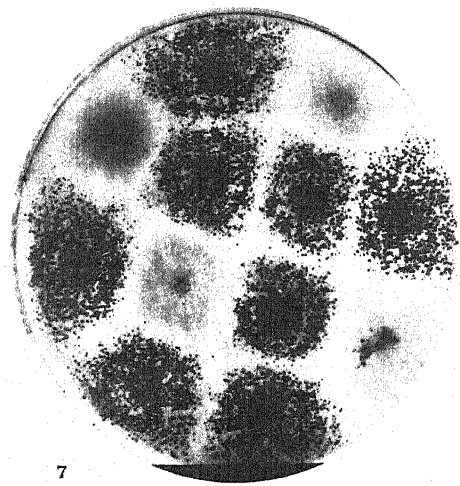
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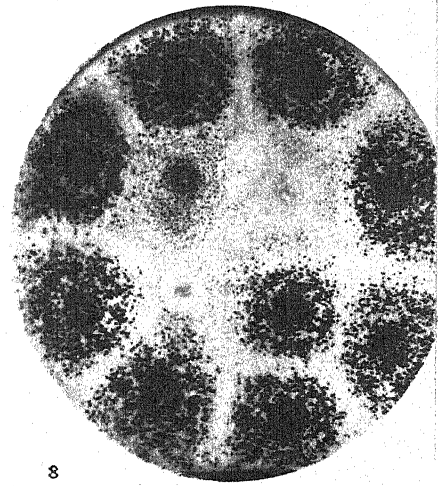
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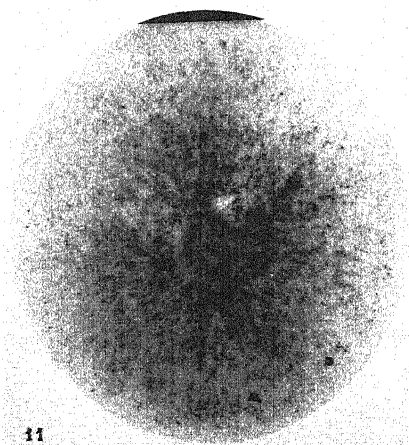
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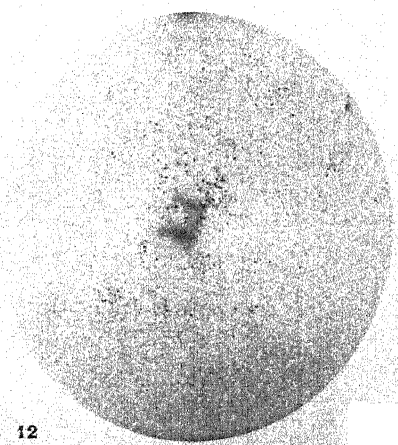
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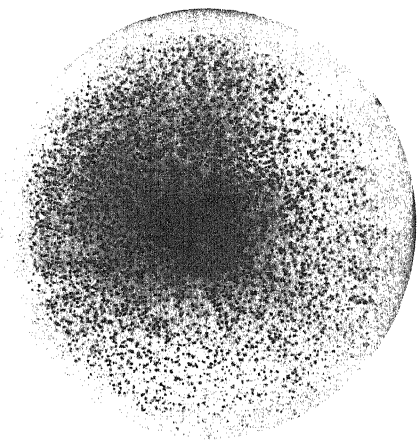


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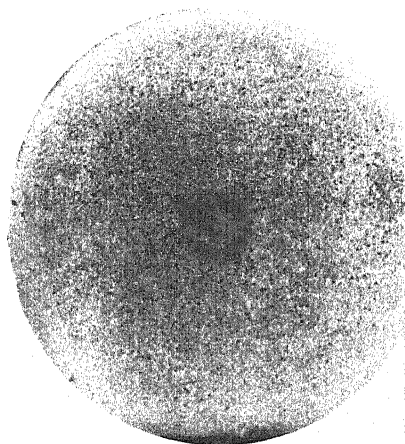


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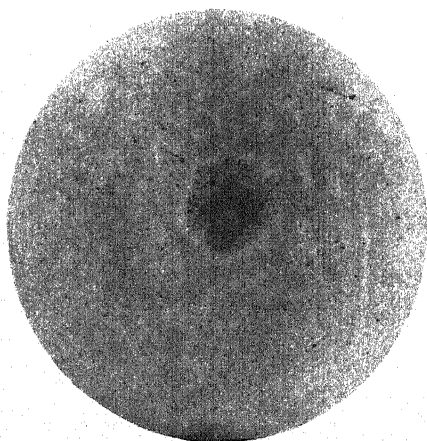




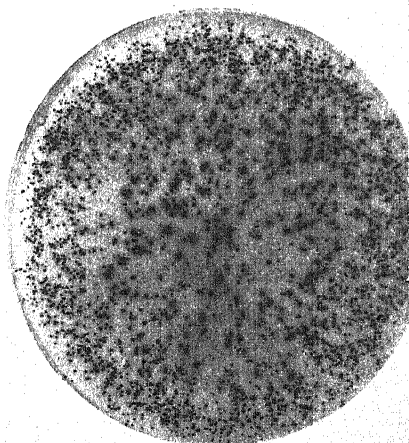
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Fig. 9. *Chaetomium cochliodes*. The parent strain.

Figs. 10-12. Pure cultures of saltants of *C. cochliodes* derived from the parent by means of X-ray treatment.

Fig. 10. Saltant No. 31. The perithecia are more numerous and smaller than in the parent, the perithecial hairs are straight and of a yellow-brown colour.

Fig. 11. Saltant No. 13. The substratum is coloured brown, the perithecia are normal but more numerous than in the parent.

Fig. 12. Saltant No. 2. The substratum is not coloured. The perithecia are smaller than in the parent and contain no asci.

PLATE XIV.

Figs. 13-16. Pure cultures of saltants of *C. cochliodes* derived from the parent by means of X-ray treatment.

Fig. 13. Saltant No. 22. The perithecia are somewhat smaller than in the parent, and the hairs are straight and lighter in colour. An increased amount of aerial mycelium is formed.

Fig. 14. Saltant No. 33. A few normal perithecia evenly scattered over the surface of the colony are produced, and also very many smaller perithecia. The smaller perithecia are sterile and have straight hairs. A larger amount of aerial mycelium is formed than is the case with the parent.

Fig. 15. Saltant No. 25. The substratum is a little darker than in the parent. The perithecia are very few in number and are evenly distributed over the surface of the colony.

Fig. 16. Saltant No. 7a. The medium is strongly coloured, and the submerged hyphae show greater coloration than is the case with the parent strain. The perithecia are not evenly distributed over the surface of the colony but at the centre occur in groups of two or three.



# Chemical Studies in the Physiology of Apples.

## XII. Ripening Processes in the Apple and the Relation of Time of Gathering to the Chemical Changes in Cold Storage.

BY

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With eleven Figures in the Text.

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### INTRODUCTION.

A LARGE number of observations during recent years have indicated that, while storage conditions are of fundamental importance in the successful handling of apples, the storage life is to a considerable extent predetermined by orchard factors (7, 37, 44, 50, 52, 42, 12). Of the many factors studied, such as climate, manurial treatment, season, age of tree, and date of gathering, especial prominence has been given to the last in its relation to the incidence of the various physiological diseases to which apples in cold storage are subject. It is now generally thought that internal browning (flesh collapse or soggy breakdown), Jonathan breakdown, and Jonathan spot are more prevalent in mature than in immature apples (9, 18, 23, 24, 28, 54, 37, 43, 44, 50), while bitter pit and scald are associated with immature fruits (12, 61, 63, 64, 40). Nevertheless marked differences of opinion have been expressed on the best time to gather apples; growers in some districts are said to gather too early (10, 28, 39, 40, 46, 59), and in others too late (18, 19, 35, 50). The importance of the pre-storage

conditions is further emphasized by the different responses to storage conditions of the Grimes apple, obtained by various American workers from different localities (24, 28, 53, 55, 56), and by Tiller (59, 60) in New Zealand, working on varieties obtained from different sources. It seems clear from the work referred to above that the degree of maturity at gathering is a very important factor in determining the subsequent behaviour of apple crops in storage and, further, that in practice crops are gathered at very different stages of ripeness in different districts. That a definite change in the metabolic rate occurs during ripening is suggested by the observations of Burroughs (10), Kidd and West (23), and of Harding (27), who found that the respiratory activity of gathered fruit rose to a maximum and then fell. Kidd and West (23) also state that the increase of carbon-dioxide output is independent of gathering, and further that there is a change in the respiratory quotient from values greater than one to values less than one during this rise to a maximum carbon-dioxide output.

Several investigations of the nature of the ripening process have been reported, but most of the data relate to size, colour, and hardness (measured by the pressure tester) (36, 37, 39, 40), and where chemical data exist these are very incomplete. The relation of chemical composition to keeping qualities has received some attention, and it is now well known that large differences occur in the composition of the fruit from season to season, orchard to orchard, and even from tree to tree (4, 36). The various observations have not revealed many definite relationships between crude chemical composition and storage qualities (4, 24, 49, 63)—Magness (37) has, in fact, stated that differences in chemical composition due to variations in growing conditions are so great in relation to those due to stage of maturity that any picking test based on chemical composition would prove unsatisfactory. It thus appears that, if chemical data are to be of use in the study of disease in storage, a detailed knowledge of the changes throughout the growing period is necessary (28), and this knowledge must be related to the subsequent changes in store. A study of the chemical history of the apple has therefore been undertaken from the time of 'petal-fall' throughout the growing period, and during a storage period (at 1° C.) of eight to ten months.

The investigation falls into two parts: firstly, a study of the changes of the fruit on the tree throughout two growing seasons, 1929 and 1930, of two varieties of apple, Bramley's Seedling and Worcester Pearmain; and, secondly, a study of the changes during storage at 1° C. of samples of the two varieties of apple gathered at different dates during the season 1929.

#### PART I. THE RIPENING PROCESS IN THE APPLE.

The maturing or ripening process in the apple is generally regarded as the final stage of fruit development, but, since the growth process is con-

tinuous from the time of fruit setting, a complete study of ripening must include observations of the changes in chemical composition from the time of petal-fall. Accordingly, analyses of the two varieties of apple have been made from the date of petal-fall until well after the grower would have gathered the crop.

A number of analyses of growing fruits has been published. These include data for grapes (14, 15, 17), oranges (16), cherries (29), melons (13, 57), figs (62), dates (21), peaches (47), and pears (30), in addition to those for the apple (4, 8, 24, 33, 46, 55). Most of these investigations deal with the period just prior to the commercial gathering date, and emphasize the marked changes in specific gravity of expressed juice and sugar-content, especially sucrose, during the period under observation. In the case of grapes and apples the decreasing concentration of acid and the progressive softening of the fruit have also been noted. None of these workers attempt to account for all the changes occurring in the fruit, or to establish any quantitative relations between the various substances accumulating during growth. In the present work on the apple, quantitative estimations have been made of as many constituents as possible throughout growth, in order to study more fully the course of the accumulation of materials in the fruit.

#### *Material for Analysis.*

Analyses have been carried out during two seasons, 1929 and 1930, of the varieties Bramley's Seedling and Worcester Pearmain grown at the Horticultural College, Swanley, in grassed-down orchards, not receiving manurial treatment. The trees were standard, and about twenty years old. Most of the samples for analysis comprised two fruits from each of 13 trees in 1929 and from each of 14 trees in 1930 in the case of Bramley's Seedling, and two fruits from each of 10 trees in 1929 and 11 trees in 1930 for Worcester Pearmain. Collections were begun when most of the bloom had fallen, and the browned stamens were still attached. The results of the determinations made in 1929 showed that during the first weeks of development the percentage of alcohol-insoluble material was very high and of sugars very low, and, in consequence, samples of about 20 fruits were insufficient to obtain reliable values of the amount of sugar present in the early stages of growth. In addition, difficulties were experienced in the estimation of fructose and glucose in the highly coloured solutions obtained by alcohol extraction of very immature apples. Before the 1930 season, therefore, satisfactory methods of estimating small amounts of sugars, and of decolorizing the solutions, were developed by Widdowson (65), in collaboration with the author. The size of the samples in the early collections of the 1930 series was such as to ensure trustworthy sugar data, and even with the improved methods needed to be rather large at the start (250 fruits from Bramley's Seedling and 108 for Worcester Pearmain). The actual

number of fruits in each sample is recorded in the Tables giving the complete results (I–IV). Two collections were made in the first fortnight of growth in 1929, and subsequent collections at intervals of ten to fourteen days until a fortnight after the commercial gathering date. In 1930 collections were made four times in the first ten days of growth, and then at weekly intervals till the middle of July, after which they were made every fortnight till the gathering time in the case of Worcester Pearmain (August 30), and till the end of September in the case of Bramley's Seedling. Samples of Bramley's Seedling were then gathered each week till the end of November, when all the fruit had fallen from the trees.

The commercial gathering date for Bramley's Seedling at Swanley in 1930 was October 21, and dropping became considerable about this time. In order to avoid discrepancies due to selection as a result of 'dropping', samples of windfalls were collected after this date in addition to the samples from the trees. Two windfalls were taken from under each tree when the normal samples were collected, and all the rest of the fallen fruit was removed each time. These samples of windfalls were analysed separately, and the results are recorded in Table IV marked W (windfall) alongside the analyses of the samples from the trees (T). No marked difference in the composition of the two samples was found, except that the windfalls seemed to be larger. A severe storm on November 4 removed more than half the fruit remaining on the trees, but subsequent samples still showed no definite change in the constituents estimated except in the final sample (November 21), which was composed of the few small fruits remaining on the trees. It has therefore been concluded that no serious errors are introduced by selection of properties due to dropping, and the mean values of the T and W samples have been used in constructing the figures.

#### *Methods of Analysis.*

The analyses consisted of estimations of total dry material, total nitrogen, fructose, glucose, sucrose, acid, and alcohol-insoluble material. The constituents of the alcohol-insoluble material, starch (when present), pectins, hemicelluloses, and cellulose were estimated by Widdowson, and her results are described in a separate paper (66).

*Dry matter* was usually determined by drying at 50° for 44 hours (2), but in some of the early samples in 1929 (marked with an asterisk in Tables I to IV) by drying at room temperature over phosphorous pentoxide *in vacuo* to constant weight.

*Total nitrogen* was determined on the dry material by the Kjeldahl method (1).

*Sugars* were estimated in solutions obtained from the alcohol extraction of fresh apple tissue by a combination of the iodimetric method with either Lane and Eynon's copper reduction method or a modification of

Hagedorn and Jensen's ferricyanide method, according to the amount of sugar present (5, 20, 32, 65).

*Acid* was determined by titration of an aliquot portion from the alcoholic extract (see below).

*Alcohol-insoluble material* was determined by weighing the residue from the alcoholic extraction for the sugar determinations (2, 32).

*Starch* was determined in the insoluble residue by hydrolysis with taka-diastase and estimation of the sugars produced (65, 66).

These methods have all been fully described elsewhere (see references), but, since the method of determination of sugars in the apple was discussed by Evans (20) and by Haynes and Archbold (32), several modifications have been introduced. The procedure for sugar determinations used for the present work is therefore set out in detail below.

When the copper reduction method is used 100 grm. of fresh apple tissue (cut up finely by hand) are soaked overnight in 200 c.c. of 95 per cent. alcohol. The cold alcohol extract is then poured off and put aside and the pulp transferred to a 100 c.c. Soxhlet apparatus. No thimble was used in the extractor, but a small filter plate covered with filter paper was placed in the bottom, covering the end of the siphon tube to prevent it being blocked by the material, and the pulp pressed down on top of it and extracted with hot alcohol for 16 hours. No neutralizing agent is added to the extracts, since tests with calcium carbonate showed that it only partially neutralized the acid in alcohol solution. Further, no difference was found in the estimated amounts of sugar in untreated solutions compared with solutions treated with calcium carbonate during the hot extraction or with ammonia during both cold and hot extractions. Hydrolysis during alcoholic extraction is therefore presumed to be negligible. The preliminary experiments (32) suggested that some hydrolysis did occur, but the later tests definitely negated this.

The two alcohol extracts are then combined and the alcohol distilled off under reduced pressure at a temperature always below 30° C. and usually about 22° C. The remaining liquid is then transferred to a 200 c.c. graduated flask and made up to volume with distilled water. Two samples of 20 c.c. are then withdrawn by means of a pipette for duplicate titrations with N/10 soda to determine acidity. 100 c.c. of the remaining liquid (equivalent to the extract from 50 grm. of apple) are transferred to a graduated litre flask and sufficient N/10 soda added to leave the solution only just acid (= 1.5 c.c. N/10 soda); it is then diluted to 900 c.c. and basic lead acetate (usually 4-5 c.c. for extracts from mature apples) added till no more precipitate is obtained. After standing for ten minutes the excess lead is removed by the addition of saturated sodium phosphate. If the basic lead acetate solution is prepared as described by Armstrong (6), the volume of phosphate required is two to three times that of the lead.

Each fresh lead solution is tested to find the amount of phosphate required to remove all the lead, and this amount is added to the apple extracts to ensure complete removal of lead. After the addition of phosphate the solution is made up to volume and filtered. Estimation of total sugars is then carried out exactly as described by Evans (20).

The cleared filtrate is generally coloured and requires decolorization by boiling with charcoal before proceeding to iodimetric estimations. In any case this process has been found necessary, even in colourless apple extracts, if sodium phosphate is used as the de-leading agent (5). 200 c.c. of the cleared solution are therefore boiled with 0.5 gm. of charcoal (Suchar)<sup>1</sup> for one minute and the solution filtered; if the solution is still coloured the process is repeated, using fresh charcoal, as often as is necessary, to obtain a colourless extract. One more boiling is required after the solution is apparently colourless (5). The colourless solution is finally filtered into a 250 c.c. graduated flask and the charcoal well washed with hot water. After cooling, the filtrate is made up to volume and the iodine value determined exactly as described by Archbold and Wid-dowson (5). The volume required for each determination is generally 75–100 c.c. of the decolorized extract. This amount usually ensures that not more than half the iodine is reduced during the reaction. Owing to the relatively large amount of fructose in the apple, the actual amount of sugar present may be greater than the usual amount of 0.08 gm. If more than half the iodine is used the determination must be repeated, using less of the sugar solution.

This method can be used with smaller quantities of apple or immature fruit containing low concentrations of sugar, provided the volume of the cleared solution is at least 500 c.c. and contains 600 mg. of sugar. This volume is sufficient to check the estimation if it be necessary.

When the sugar concentration is too low or there is only a little material available, the modified Hagedorn and Jensen method was used. The available weight of apple tissue was extracted, and the extract cleared and decolorized as described above. The final volume of the cleared and decolorized solution was chosen so that the concentration of sugar was not more than 3 mg. in 5 c.c. Iodimetric and ferricyanide oxidations were carried out on the colourless extract exactly as described by Wid-dowson (65).

The extracts from fruit in the very early stages of growth are often very highly coloured, and in some cases it was necessary to boil with charcoal as many as ten times before obtaining a colourless extract. The sugar values in these cases were a little low, owing to loss of sugar during boiling (5). Generally one to three boilings were sufficient. Much more basic lead acetate is also required for clearing these extracts than for those

<sup>1</sup> Suchar is a specially prepared active charcoal supplied by the British Suchar Processes, Ltd.



from mature apples; up to 19 c.c. for the extract from 50 grm. of apple has been found necessary.

The values in the tables for fructose and glucose were obtained by solving the simultaneous equations derived from the two determinations of reducing power (5, 65). For the estimations using copper reduction a special table was constructed giving the volume of solutions of different concentrations of fructose and glucose always in the ratio 3 : 1, reduced by 10 c.c. of Fehling's solution under Lane and Eynon's conditions. Sucrose was determined as the difference between reducing sugar and total sugar obtained from the table, and is expressed as true sucrose ( $C_{12}H_{22}O_{11}$ ). Corrected values of total sugars were then obtained as the sum of fructose, glucose, and sucrose. Use of the table for invert sugar introduces an error of about 3 per cent. when the proportions of the two sugars present are as unequal as is the case in the apple. When the ferricyanide method was used, fructose and glucose were estimated separately before and after hydrolysis of the sucrose, so it was only necessary to calculate the difference as true sucrose.

## RESULTS OF THE ANALYSES OF DEVELOPING APPLES.

### *Concentration Changes during Growth.*

The results of the analyses of the two varieties of apple during growth in 1929 and 1930 are shown in Tables I to IV, in terms of percentage of fresh weight at time of analysis, and thus show concentration changes throughout growth. One set of results (Bramley's Seedling 1930) is shown graphically in Fig. 1. The same types of curves are obtained from the data for 1929 and for the Worcester Pearmain, although the relative amounts of the constituents differ in the two varieties, and the scatter of the observations is greater in 1929 than in 1930 in both cases.

It will be seen that there is no starch present during the first three weeks of development in either variety, and that the maximum values for starch occurred in Worcester Pearmain about July 29, 1929, 2.03 per cent.; and about July 30, 1930, 2.01 per cent.; and in Bramley's Seedling two to three weeks later, August 14, 1929, 1.21 per cent.; and August 26, 1930, 1.46 per cent. After these dates the concentrations fell. In Worcester Pearmain some starch remained at the gathering time in both seasons, while in Bramley's Seedling starch disappeared about the gathering time (October 10-21) in both 1929 and 1930. The growing period falls conveniently into three phases limited by the appearance and disappearance of starch. The first is up to the appearance of starch (about three weeks, end of May to middle of June), the second is the period of starch synthesis (five to six weeks in Worcester Pearmain, middle of June to end of July, and eight to ten weeks in Bramley's Seedling, middle of June to end of August), and the third the period of starch hydrolysis (end of July until

the picking date in Worcester Pearmain, usually early September, and about six weeks in Bramley's Seedling, middle or end of August to middle of October).

*Increase in Weight during Growth.*

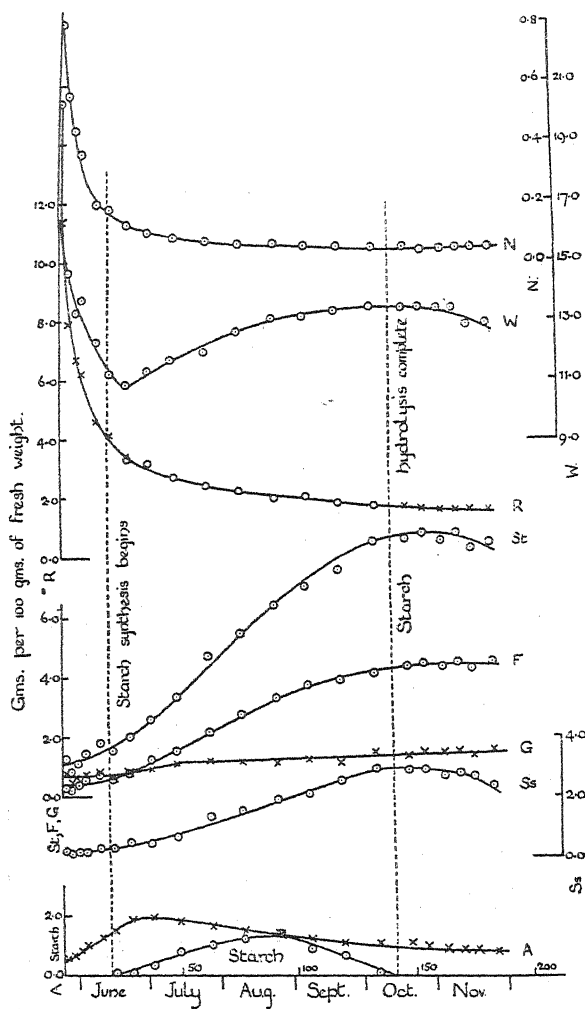


FIG. 1. Concentration of the constituents of the pulp of Bramley's Seedling apples during growth. May–November, 1930. (Growers' gathering date October 21). Total nitrogen, N, dry weight, W, alcohol-insoluble material other than starch, R (observations denoted by circles during the time starch is present and by crosses when starch is absent). Total sugar, ST, fructose, F, glucose, G, sucrose, Ss, acid, A, and starch.

The increase during growth was determined by weighing each sample before it was cut up for analysis (see Tables I–IV).

In both seasons the Worcester Pearmain apples were increasing rapidly in size when the crop was gathered. In 1929 the Bramley's Seedling were still increasing in size when gathered on October 22; this was a fortnight after the grower gathered the main crop. In 1930 the fruit was allowed to remain on the trees and growth stopped abruptly at the end of October (see Fig. 2). In this season the grower gathered on October 21st at the maximum size. It appears that, in general, apples are gathered before they have reached the maximum size. That this practice is fairly widespread is supported by the work of Carne, Pittman, and Elliott (12) in Australia, and of several American observers

(36, 39, 40, 46, 55). The fact stated above that fruit may remain on the tree a month after growth has ceased is in opposition to the statement of some

TABLE I.  
*Analyses of Worcester Pearmain Apples during Growth. May-September 1929.*

Results expressed as percentages of fresh weight. No. of fruits per sample, 20.

Date of Analysis.	Days from May 25.	Mean wt. per apple. gm.	Dry wt. W	Total Sugar. St	Sucrose $C_{12}H_{22}O_{11}$ Ss	Fructose. F	Glucose. G	Acid as Malic. A	Alcohol-insol. residue (other than Starch.) R	Starch.	Total Nitro-gen. N	(St + R + A + starch) as p.c. of dry wt.
4. 6. 29	11	0.75	*16.86	—	—	—	—	1.07	8.10	None	0.137	—
15. 6. 29	22	1.71	*15.06	—	—	—	—	1.21	6.24	0.342	0.178	—
26. 6. 29	33	5.16	*15.84	4.46	0.33	2.05	2.08	1.48	4.71	1.766	0.100	72.10
5. 7. 29	42	11.45	*13.61	4.53	0.66	2.07	1.80	—	4.55	0.911	0.064	—
17. 7. 29	54	19.5	17.15	6.95	0.56	5.03	1.36	1.09	5.23	1.766	0.055	87.70
29. 7. 29	66	28.5	17.04	7.55	1.14	4.61	1.80	0.91	4.53	2.031	0.038	88.15
14. 8. 29	81	45.0	17.20	9.60	1.71	6.46	1.43	0.73	3.73	1.899	0.024	92.79
26. 8. 29	93	49.7	17.15	10.75	1.98	6.74	2.03	0.68	3.08	0.964	0.023	90.20
17. 9. 29	115	72.2	16.99	12.15	1.96	8.24	1.95	0.37	2.41	0.477	0.024	90.70

\* Dried over  $P_2O_5$  in vacuo.

TABLE II.  
*Analyses of Bramley's Seedling Apples during Growth. May–October 1929.*  
 Results expressed as percentages of fresh weight. No. of fruits per sample, 26.

Date of Analysis.	Days from May 25.	Mean wt. per apple. gm.	Dry wt. W	Total Sugar. St	Sucrose $C_6H_{12}O_{11}$ . Ss	Fructose. F	Glucose. G	Acid as Malic. A	Alcohol-insol. residue (other than Starch). R	Starch.	Total Nitro-gen. N	(St + R + A + starch) as p.c. dry wt.
25. 5. 29	1	0.15	*20.19	—	—	—	—	—	17.64	None	0.671	—
4. 6. 29	11	0.47	*15.54	—	—	—	—	1.05	7.91	"	0.314	—
15. 6. 29	22	2.66	*12.25	—	—	—	—	2.26	4.88	0.369	0.174	—
26. 6. 29	33	7.69	*11.65	3.41	0.29	1.60	1.52	2.13	3.45	0.397	0.095	80.60
5. 7. 29	42	19.58	*11.48	3.42	0.58	1.39	1.45	—	3.34	0.620	0.067	—
17. 7. 29	54	39.0	12.16	4.22	0.45	2.12	1.65	2.18	3.33	0.701	0.059	85.77
29. 7. 29	66	56.0	12.92	5.56	1.03	2.97	1.56	1.78	3.43	0.930	0.050	90.56
14. 8. 29	81	61.2	13.75	6.64	1.44	3.59	1.61	1.57*	2.95	1.213	0.034	89.96
26. 8. 29	93	62.3	12.98	6.92	1.42	3.82	1.68	1.36	2.69	1.014	0.028	92.30
10. 9. 29	108	92.3	13.77	8.53	1.60	4.98	1.95	1.24	2.43	0.690	0.026	93.61
26. 9. 29	124	94.1	14.20	9.73	1.65	5.69	2.39	1.15	2.17	0.370	0.026	94.51
10. 10. 29	138	108	14.44	10.22	2.42	5.88	1.92	1.13	—	—	0.029	95.43
22. 10. 29	150	118	14.42	10.14	2.80	5.73	1.61	1.07	2.08	0.035	0.028	92.44

\* Dried over  $P_2O_5$  in *vacuo*.

TABLE III.

*Analyses of Worcester Pearmain Apples during Growth. May-August 1930.*

Results expressed as percentages of fresh weight.

Date of Analysis.	Days from May 27.	No. of fruits per sample.	Mean wt. per apple, gram.	Dry wt. W.	Total Sugar, St	Sucrose $C_{12}H_{22}O_{11}$ , Ss	Fructose, F	Glucose, G	Acid as Malic, A	Alcohol-insol. residue (other than Starch), R	Starch.	Total Nitrogen, N	(St + R + A + starch) as p.c. dry wt.
27.5.30	1	108	0.048	22.25	1.66	0.48	0.32	0.86	0.787	14.63	None	0.624	76.76
30.5.30	4	110	0.10	15.97	1.24	0.11	0.48	0.65	0.803	10.17	"	0.576	76.46
2.6.30	7	114	0.21	14.91	1.23	0.15	0.46	0.62	0.827	8.80	"	0.482	72.84
5.6.30	10	55	0.50	14.10	1.41	0.18	0.56	0.67	0.967	7.59	"	0.386	70.71
11.6.30	16	23	1.78	13.38	1.55	0.22	0.50	0.83	1.214	6.20	"	0.274	66.97
17.6.30	22	22	4.28	12.66	1.98	0.20	0.75	1.03	1.269	5.68	"	0.206	70.54
24.6.30	29	22	6.20	13.05	3.10	0.64	0.96	1.50	1.280	5.00	0.21	0.162	73.49
3.7.30	38	22	11.22	13.74	4.48	0.44	2.20	1.84	1.291	4.60	0.89	0.122	81.95
14.7.30	49	22	21.77	14.74	5.81	0.78	3.23	1.80	1.103	4.04	1.68	0.091	85.69
28.7.30	63	22	38.00	14.80	7.13	1.30	4.40	1.43	0.818	3.45	2.01	0.050	90.61
11.8.30	77	21	56.57	15.52	8.63	1.97	5.46	1.20	0.637	3.10	1.96	0.035	92.33
26.8.30	92	22	72.64	16.28	10.51	2.85	6.39	1.27	0.600	2.88	1.29	0.026	93.86

TABLE IV.  
*Analyses of Branley's Seedling Apples during Growth. May–November 1930.*  
*Results expressed as Percentages of Fresh Weight.*

Date of Analysis.	Days from May 27.	No. of fruits per sample.	Mean wt. per apple. gram.	Dry wt. W	Total Sugar. St	Sucrose $C_{12}H_{22}O_{11}$ Ss	Fructose. F	Glucose. G	Acid as Malic A	Alcohol-insol. residue (other than Starch). R	Starch.	Total Nitro-gen. N	(St + R + A + starch) as p.c. dry wt.
27.5.30	1	250	0.11	19.35	1.27	0.19	0.27	0.81	0.670	11.38	None.	0.814	68.84
30.5.30	4	154	0.24	13.72	0.83	0.10	0.22	0.51	0.716	7.96	"	0.568	69.31
2.6.30	7	76	0.57	12.37	1.16	0.17	0.41	0.58	0.877	6.75	"	0.454	71.06
5.6.30	10	40	0.96	12.79	1.50	0.17	0.56	0.77	1.056	6.15	"	0.366	68.10
11.6.30	16	29	2.23	11.35	1.87	0.30	0.74	0.83	1.314	4.06	"	0.201	69.07
17.6.30	22	29	5.77	10.24	1.58	0.31	0.58	0.69	1.572	4.06	0.118	0.179	71.76
24.6.30	29	42	13.31	9.90	2.06	0.47	0.77	0.82	1.915	3.35	0.124	0.127	75.25
3.7.30	38	28	24.88	10.36	2.60	0.42	1.24	0.94	2.001	3.20	0.377	0.104	78.86
14.7.30	49	28	37.64	10.72	3.37	0.70	1.55	1.12	1.849	2.75	0.840	0.0850	82.18
28.7.30	63	28	54.35	11.00	4.73	1.37	2.16	1.20	1.722	2.50	1.031	0.0713	90.73
11.8.30	77	28	78.50	11.72	5.51	1.54	2.77	1.20	1.568	2.29	1.261	0.0578	90.70
26.8.30	92	26	87.94	12.12	6.41	1.93	3.32	1.16	1.424	2.05	1.463	0.0560	93.56
8.9.30	105	28	101.89	12.16	7.05	2.09	3.74	1.22	1.276	2.11	0.912	0.0503	93.34
22.9.30	119	28	123.10	12.36	7.61	2.54	3.90	1.17	1.146	1.89	0.700	0.0456	91.83
10.10.30	134	28	138.17	12.52	8.55	2.93	4.10	1.52	1.166	1.82	0.135	0.0449	93.29
21.10.30	148	28	150.68	12.46	8.62	2.91	4.37	1.34	1.156	1.73	None	0.0447	92.38
28.10.30	155	T. 26	134.65	12.44	8.81	2.93	4.34	1.54	1.039	1.68	"	0.0405	92.68
"	"	W. 26	145.88	12.50	8.92	2.89	4.57	1.46	1.002	1.66	"	0.0417	92.64
5.11.30	163	T. 26	139.07	12.44	8.79	2.80	4.50	1.49	0.800	1.69	"	0.0436	92.12
"	"	W. 29	141.14	12.40	8.37	2.66	4.20	1.51	0.688	1.64	"	0.0499	88.71
13.11.30	170	T. 26	133.2	12.34	8.67	2.71	4.42	1.54	0.971	1.66	"	0.0511	91.57
"	"	W. 24	140.6	12.56	9.01	2.95	4.53	1.53	0.978	1.63	"	0.0477	92.52
19.11.30	176	T. 23	139.30	11.82	8.16	2.59	4.17	1.40	0.978	1.66	"	0.0547	91.37
"	"	W. 25	143.96	11.92	8.69	2.83	4.43	1.43	0.921	1.69	"	0.0445	94.80
"	"	T. 18	111.8	12.06	8.61	2.45	4.51	1.65	0.899	1.65	"	0.0501	92.54
27.11.30	184	W. 17	103.7	11.80	8.44	2.33	4.56	1.55	0.831	1.66	"	0.0465	92.63

of these workers that growth continues as long as fruit remains attached to the tree. Very few leaves remained on the trees when the final sample was collected and, in view of the important influence of the leaves on the growth of the fruit (26), it seems possible that growth may cease when the leaves no longer supply material.

Worcester Pearmain are generally gathered some weeks earlier than Bramley's Seedling, although fruit of the two varieties sets at the same time. In both cases starch appeared 22 days after the blossom had fallen, but the time taken to reach the starch maximum was less in Worcester Pearmain than in Bramley's Seedling, so that the growth cycle is probably shorter in Worcester Pearmain. In 1930 the total growth period in Bramley's Seedling was 150 days, and the maximum starch concentration was reached after 90 days, or three-fifths of the total time. The starch maximum was reached in 80 days in Worcester Pearmain, so that if this represents the same proportion of the total growth period, as in Bramley's Seedling, growth would cease after 134 days or 16 days earlier than in Bramley's Seedling. The fruit was actually gathered after 92 days, or 58 days before Bramley's Seedling, and was thus in a very immature condition from the physiological standpoint.

The mean values of the weight of fruit at each collection are shown in Figs. 2 *a* and 2 *b*. The date of the first appearance of starch is marked by dotted lines parallel to the ordinate. In all four cases the rate of growth increases until starch appears (three weeks after blossom-fall) and then remains constant throughout the rest of development.

The form of the growth curve for fruits has been considered by Copeman (16), who measured growth by increase in weight of oranges, and by Gustafson (25), who measured increase in volume of tomatoes. Both these workers favoured the autocatalytic expression to express the growth process. Although the rate of growth of the apple undoubtedly increases at first, the later parts of the curves of Fig. 2 cannot be represented by an autocatalytic expression. In the case of ripening oranges, where fruits, even off the same tree, ripen at very different times, the apparent falling off in rate observed by Copeman might be due to the inclusion in the later samples of some fruits which had ceased growing. Some preliminary work (4), in which measurements were made on samples of ten apples at rather infrequent intervals, also suggested that the growth rate might fall at the end of the growth period. The evidence now obtained, however, seems to establish that growth rate (measured by weight) of the apple is constant after starch synthesis begins. If allowance could be made for transpiration losses, as suggested by Copeman (16), the rate would probably increase a little. Where increase in volume is used as a measure of growth, the increasing volume of the air spaces must be taken into account. The change in specific gravity will then be a factor in determining the difference

between the form of the curves for weight increase and volume increase. The latter might well have the S-shaped form found by Gustafson.

In order to obtain the best estimates of the mean weight at any date, straight lines of closest fit have been calculated by the method of least

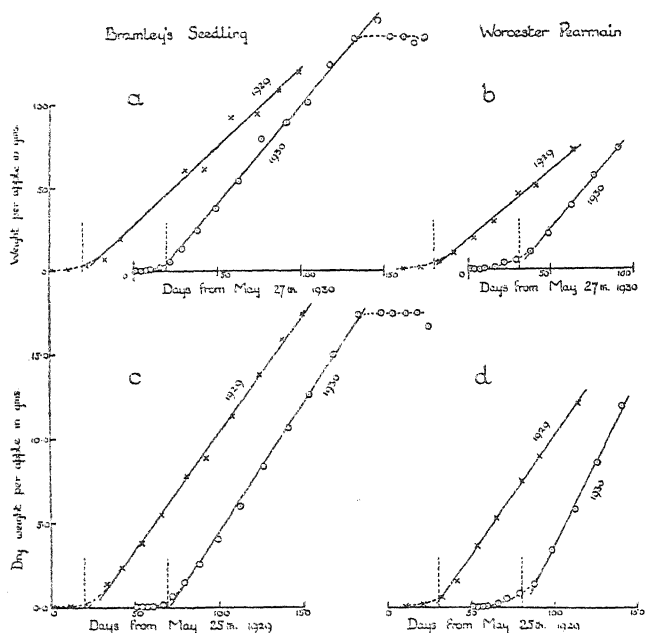


FIG. 2. Bramley's Seedling and Worcester Pearmain apples during growth. 1929 and 1930. *a* and *b* the mean weights per apple, and *c* and *d* the total solids, pulp only, per apple. Vertical dotted lines mark the date of the first appearance of starch.

squares for the observations made subsequent to the appearance of starch. In the case of Bramley's Seedling 1930 the observations after October 22 have been omitted from the calculations, as there was clearly no increase in weight after that date. In Fig. 2 the calculated lines are drawn through the observations, and the early parts not represented by the lines are shown as dotted lines.

#### *Increase in Total Solids during Growth.*

The concentration of total solids falls rapidly during the first phase of growth—in the case shown in Fig. 1, from 19.35 per cent. to 10.24 per cent. in 22 days. The concentration then rises during starch synthesis, but at a decreasing rate, and during the last phase the increase in total solids concentration is very small.

The calculated values of the weights of the apple at each date obtained from the equations to the lines of Fig. 2 *a* and *b* have been used to obtain



measures of the total weight per apple of each constituent from the concentration data of Tables I-IV. It is, of course, realized that a small error is introduced by this procedure, since the weight of the apple includes the core and skin, while the estimations are of the constituents of the pulp only. The values obtained for total dry matter are shown in Fig. 2 *c* and *d*, from which it is clear that the rate of intake of total solids increases rather rapidly until starch synthesis begins, and then remains approximately constant.

The average rates of increase for the period subsequent to the appearance of starch, were therefore, found by calculating the equations for the straight lines of closest fit for the observations, and these straight lines are drawn in Fig. 2 *c* and *d*. The average rates of increase of total weight and total solids are shown in Table V.

TABLE V.

*The Average Rates of Increase of Total Weight and Total Solids of Bramley's Seedling and Worcester Pearmain during Growth.*

		Average rate of total growth. gm. per apple per day.	Average rate of solid intake.	Ratio <i>b/a</i>
		<i>a</i>	<i>b</i>	
Bramley's Seedling	1929	0.931	0.138	0.148
	1930	1.172	0.150	0.128
Worcester Pearmain	1929	0.806	0.140	0.174
	1930	1.161	0.193	0.166

The rates of total growth were higher in 1930 than in 1929 in both varieties, but the ratios of solid intake to total growth were lower, and in consequence the crop was larger in 1930, but the final solid concentration was lower. The ratio of solid to total intake is higher in Worcester Pearmain than in Bramley's Seedling, resulting in a higher final concentration of total solids in these apples.

If it is assumed that both rate of total intake and solid intake are constant after starch synthesis begins, the concentration of solids at any time will be given by the expression

$$C = \frac{a + bt}{A + Bt}$$

where ( $A + Bt$ ) and ( $a + bt$ ) represent the total weights and total solids per apple at any time  $t$ . The concentration of solids will rise as  $t$  increases, gradually approaching a limiting value of  $b/B$ , and the rate of change of concentration will diminish as this value is approached. That this is a close approximation to the facts is seen in Fig. 1, where the concentration of total solids (Bramley's Seedling 1930) rises at a decreasing rate after

starch synthesis has begun, and the change in concentration in the last month of growth is only from 12.36 per cent. to 12.46 per cent.

If, however, the concentrations are calculated as above for each date, it is found that the values obtained for the early stages of growth are too high and for the later stages too low, which would be the case if the rate of intake of solids were really increasing slightly. This suggestion is supported by the position of the observations relative to the calculated lines in Fig. 2 *c* and *d*, where the first and last observations tend to lie above the lines and the middle ones below.

By plotting logarithms of the observations it can be seen that both total weight and total solids increase according to an exponential law up to the time starch synthesis begins, that is, during the first three weeks of growth.

#### *Increase in Sugars during Growth.*

The initial concentration of sugar is only about 1 per cent. in all four sets of apples and at the end of the first phase of growth, although the rate of intake is rising, the concentration only reaches 1.59 to 2.5 per cent. Glucose is present in larger amounts than either fructose or sucrose. The amount of each sugar per apple (obtained in the same way as the dry matter per apple) increases according to the exponential law until starch synthesis begins. During the second phase, sugar concentration increases rapidly at a nearly constant rate, and the large excess of fructose characteristic of the mature fruit is accumulated, while glucose concentration either remains constant or increases slightly. Fructose concentration increases at a declining rate and sucrose at an increasing rate (see Fig. 1). The rate of increase in total sugar concentration remains nearly uniform or decreases slightly from the first appearance of starch until starch has practically all gone. In Bramley's Seedling the sucrose concentration does not reach a maximum value until just before starch disappears and then remains constant until starch is gone. In Worcester Pearmain, however, in 1929 a maximum concentration was reached while there was still nearly 1 per cent. of starch in the fruit. The sucrose concentration then remained constant for three weeks before gathering; and the only increase in sugar concentration was in fructose (see also p. 439). The crop in 1930 was picked before this stage was reached (see Fig. 3). It may be that in the less acid apples hydrolysis of sucrose begins to exceed synthesis at an earlier stage of maturation, or the glucose produced from starch may all be converted to stable fructose and sucrose synthesis inhibited.

The concentration changes of sucrose, fructose, acid and, starch during development of Worcester Pearmain in 1929 and 1930 are shown in Fig. 3 *a* and *b*, and the amounts of sugar per apple in Bramley's Seedling 1930 in Fig. 4 (cf. also Fig. 1). From Fig. 1 it will be seen that a maximum

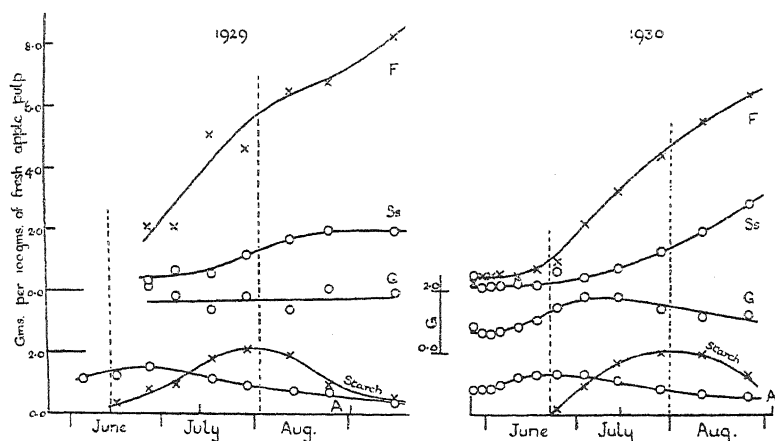


FIG. 3. Concentration of fructose, F, sucrose, Ss, glucose, G, acid, A, and starch in Worcester Pearmain apples during growth in years 1929 and 1930. Dotted lines show the dates of the first appearance of starch, and of the beginning of starch hydrolysis.

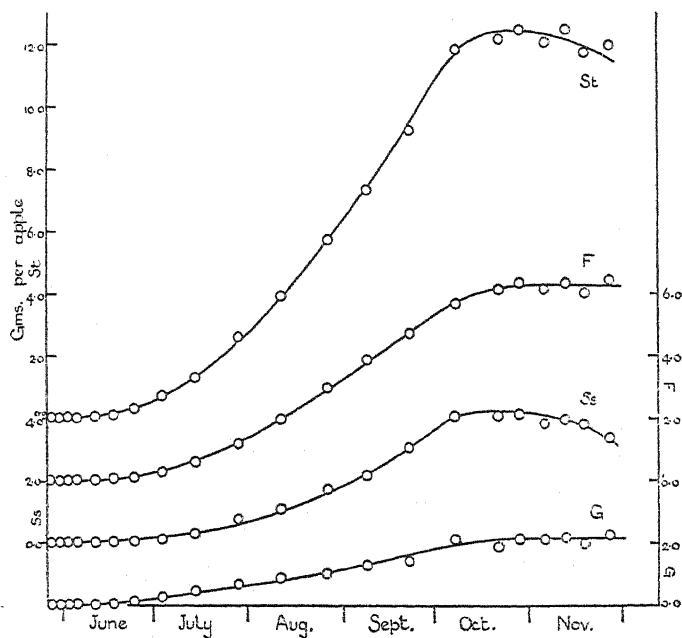


FIG. 4. The amounts of total sugar, fructose, sucrose, and glucose per apple in Bramley's Seedling during growth, 1930.

concentration of total sugar is reached just as starch finally disappears and growth ceases, which in this case was about the normal gathering time. In the later samples picked after growth had ceased, the sugar concentration remained constant for a while and finally fell off slowly. Fructose and glucose concentration also remained constant, while sucrose decreased slowly after starch disappearance. From Fig. 4 the rate of accumulation of total sugar per apple is seen to increase markedly until starch reaches the maximum value and then it remains constant or may decrease slightly. During the last stage of ripening, that of starch hydrolysis, 80 to 90 per cent. of the material entering the apple is stored as sugar, so the curve representing sugar intake will be nearly parallel to that representing total solid intake at this stage, that is, approximately a straight line.

It is generally stated that starch hydrolysis in the apple fruit produces a marked increase in sugar concentration, particularly in sucrose. From the data put forward here, which include the period of starch synthesis as well as of starch hydrolysis, it is clear that the sugar produced by starch is not sufficient to cause a marked fluctuation in the curves representing sugar change. The amounts of starch hydrolysed and the total sugar and sucrose accumulated from the time of the maximum content of starch until the commercial gathering date are given in Table VI, which shows that the sugar produced from starch is only a small fraction of the total sugar accumulated.

TABLE VI.

*Loss of Starch and Gain of Sugar in Bramley's Seedling and Worcester Pearmain during Ripening.*

		Period from fruit setting. Days.	Starch lost.	Total sugar gained. gm. per apple.	Sucrose gained.
Worcester Pearmain	1929	81-115	0.48	4.43	0.65
	1930	77-92	0.14	2.87	0.96
Bramley's Seedling	1929	93-153	0.69	7.56	2.42
	1930	92-134	1.29	6.38	2.36

The rate of increase of glucose was remarkably similar in all four sets of apples (about 0.015 gm. per apple per day), although the rate of total increase was higher in both varieties in 1930 than in 1929. The extra sugar accumulated in 1930 was fructose in Worcester Pearmain and sucrose in Bramley's Seedling.

*Increase of Alcohol-insoluble Material, Acid, and Nitrogen.*

The concentration of alcohol-insoluble material (other than starch) falls continuously throughout the growing period (see Fig. 1). The rate of fall is very marked at first and then decreases, and in the later stages of

maturation the change in concentration is very small.<sup>1</sup> Acid concentration rises during the first three weeks (see Figs. 1 and 3) and reaches a maximum value just after the appearance of starch. The maximum value is about 2 per cent. in Bramley's Seedling and about 1.3 per cent. in Worcester Pearmain. It has been suggested in an earlier paper (4) that the maximum concentration of starch and acid occur about the same time, but it is quite clear from these more detailed results that the acid maximum occurs considerably earlier than that of starch. When starch synthesis begins acid concentration falls and continues to fall at a decreasing rate throughout the rest of the growing period. Copeman (15) has reported a similar initial rise followed by a continuous fall in the acid concentration of the developing grape.

The concentration of nitrogen falls continuously, and from Fig. 1 it can be seen that the curve representing change in nitrogen concentration bears a close relationship to that representing insoluble material; further, after starch synthesis begins the curve representing acid concentration is also nearly parallel to the other two. The amounts of these three constituents accumulated thus appear to be related to one another, and in contrast to sugar accumulation, where the rate of intake per apple increases throughout growth, after the first few weeks the rates of increase per apple of these constituents fall.

The rates of increase in grams per apple per day of acid, insoluble material, and nitrogen for Bramley's Seedling and Worcester Pearmain 1930 are shown in Fig. 5. The maximum rates of increase of acid and insoluble material are reached just as starch synthesis begins, while nitrogen increase reaches its maximum value a little earlier. The decline in rate of increase of these substances is accompanied by the synthesis of starch, which thus seems to be formed alternatively to acid and insoluble material as the fruit develops. It is evident from the increasing concentration of acid before starch appears (see Figs. 1 and 3) that acid is being formed relatively faster than insoluble material at this stage, and it is found that the ratio of acid formed to insoluble material formed rises in Bramley's Seedling from 0.10 to 0.75 and in Worcester Pearmain from 0.12 to 0.25. During the period of declining rate of increase the ratio of acid to insoluble material accumulated remains practically constant at the maximum value.

The formation of cell-wall substance is thus accompanied by formation of increasing amounts of organic acids, during the first phase of growth, suggesting that these materials are alternative condensation and oxidation products of a part of the entering carbohydrate, which is presumably some form of sugar. This view is further supported by the fact that in the sub-acid Worcester Pearmain a high percentage of insoluble material is stored and in the highly acid Bramley's Seedling a low

<sup>1</sup> For a discussion of the nature of the alcohol-insoluble material see Widdowson (66).

one. It may also be pointed out that the hemicelluloses, which are being formed at this stage (66), are considered to be produced in a cell where condensation and oxidation are both proceeding rapidly (48). The forma-

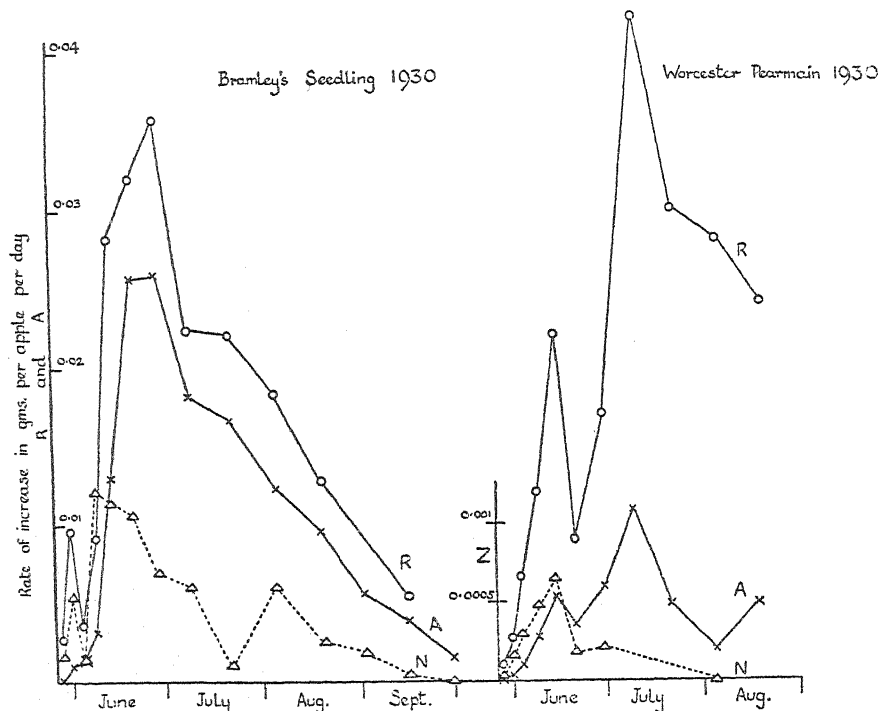


FIG 5. Rates of increase (gm. per apple per day) of alcohol-insoluble material other than starch, acid, and total nitrogen in Bramley's Seedling and Worcester Pearmain apples during growth, 1930.

tion of some acid by oxidation before condensation can occur, or by oxidation of a condensation product, would not, therefore, be unlikely. If acid does prove to originate in this way it may be the product of oxidation of  $\beta$  gluco-pyranose, which is regarded as the basic molecule of cellulose.

The relationship of nitrogen accumulation to cell-wall and acid formation is more difficult to define, partly because the nitrogen partition was not determined, and partly because increasing amounts of the nitrogen entering the fruit must be stored in the seeds, which contain about 17 per cent. of nitrogen when mature, compared with about 0.04 per cent. in the pulp. From these data, however, it is clear that the early stages of cell-wall formation are accompanied by a high rate of nitrogen accumulation and acid production, and, further, that during the later stages, when small amounts of cell-wall material are being continuously laid down, there is simultaneously a low rate of accumulation of nitrogen and acid.

Ruhland and Wetzel (58) have put forward the view that ammonium

salts of organic acids play the same part in acid-producing plants as asparagine in 'non-acid' plants. They suggest that the salts of organic acids are produced by de-amination of amino acids and the ammonia is used either at once, or later by liberation from the ammonium salt of the acid produced for protein synthesis. In the case of the apple there is no doubt that protein synthesis in the fruit and acid production are going on simultaneously, but the relationship of acid production to cell-wall synthesis seems to be closer than its relation to nitrogen (see Fig. 5). Further, in both Bramley's Seedling and Worcester Pearmain, in spite of their widely different acid contents when mature, if it is assumed that the nitrogen and acid stored in the pulp are end-products of the same reaction, there is too little acid accumulated in the first two weeks of growth, and far too much after this time, for acid and nitrogen to be derived from the same amino acid. Some of the nitrogen produced by de-amination may, of course, be stored in the seed and then no molecular relationship could be expected between acid and nitrogen accumulated in the pulp. Again, some acid may enter the fruit, as such, from the spur. No critical evidence is therefore available here to test Ruhland and Wetzel's hypothesis. Nevertheless their view that acid arises at places of rapid growth in relation to synthetic processes, rather than as an intermediate in carbohydrate respiration, is supported by these data, but it remains undecided whether the source is amino acids or sugars in process of condensation.

The estimated constituents do not account for the whole of the dry matter accumulated in the apple. The amounts accounted for by sugar, starch, insoluble material, and acid in terms of percentage of dry weight are shown in the final columns of Tables I to IV. The nitrogen has not been included, since it is known that in the early stages only about one-third, or later about one-fifth at most is soluble in alcohol, and will, therefore, not be included in the weight of insoluble residue. Assuming such soluble nitrogen is protein it would only account for about 5 per cent. of the dry weight at the beginning of the growing period and about 0.5 per cent. at the end. Up to the beginning of starch formation about 30 per cent. of the dry matter is in some form not estimated; during starch synthesis and hydrolysis this amount decreases until in the mature apple between 7 and 8 per cent. only is not accounted for. This suggests the accumulation of some substance not estimated at a decreasing rate during growth.

Since the sugar of translocation is not known with any certainty, only very tentative suggestions as to the nature of this substance can be made. Glucose is generally regarded as being very readily condensed, and the precursor of cellulose and hemicellulose. If it is assumed that sucrose is the substance entering the apple, then if glucose is used in this way, the corresponding amount of fructose produced by hydrolysis of the entering sucrose must either be converted to glucose, and condensed, or stored in

some other form, possibly a fructose polymer. When starch appears, and the rate of formation of celluloses and hemicelluloses falls, the rate of accumulation of fructose rises, while the percentage of the total material taken in which is not accounted for declines. Alternatively, tannins, glucosides, or fructosides, may be present. After starch synthesis ceases, the sugar and acid account for all but about 5 per cent. of the increase in dry weight of the fruit.

From the foregoing description of the chemical changes during growth it is evident that in the early stages of growth most of the carbon entering the pulp of the apple fruit is stored in an insoluble form. When the fruit sets, 60 per cent. to 70 per cent. of the dry material is alcohol-insoluble, and only 6 per cent. to 8 per cent. is sugar; as growth proceeds the amount stored in an insoluble form gradually decreases, and at maturity 70 per cent. of the dry material of the pulp is sugar and only 14 per cent. insoluble material. The first products of carbohydrate synthesis in the period before starch appears belong to the cellulose and hemicellulose group, which are believed to contain  $\beta$  gluco-pyranose as the basal molecule. If this is the case, this sugar must be formed in large quantities, whatever the type of translocatory sugar may be, since relatively small amounts of sugar are stored as such at this stage. When starch appears there is a sudden fall in the rate of synthesis of celluloses and hemicelluloses. Starch therefore appears to be formed alternatively to these substances, and at the same time fructose accumulates. Since  $\alpha$  gluco-pyranose is considered the basal molecule of starch, as growth proceeds the entering sugar, instead of being converted to  $\beta$  gluco-pyranose, would tend more and more to be converted to  $\alpha$  gluco-pyranose, and to fructo-pyranose. Approximately twice as much fructose as starch is formed during the period of starch synthesis. Finally, when starch synthesis ceases, over 50 per cent. of the sugar is stored as fructose.

The percentage of the total dry matter entering the pulp which is stored in the various forms, during the three phases, before starch appears, during starch synthesis, and during starch hydrolysis, are shown in Table VII.

From Table VII (opposite) it appears that the sum of the percentages of incoming material stored as acid and insoluble material is remarkably similar in the two sets of apples throughout development. Less is stored as acid and more as 'cellulose and hemicellulose' in Worcester Pearmain than in Bramley's Seedling.

The percentage stored as sugar is also nearly the same in the two varieties, but is probably a little higher in the last two stages of growth in Worcester Pearmain. The values for starch and sugar in the third phase are not strictly comparable, as starch hydrolysis was not complete in the Worcester Pearmain. If sugar produced by starch hydrolysis is subtracted, the resulting value for sugar stored is higher in Worcester Pearmain.



TABLE VII.

*Amounts of Sugar, Acid, and Alcohol-Insoluble Material Accumulating in Bramley's Seedling and Worcester Pearmain Apples during Growth, 1930.*

Bramley's Seedling.			Worcester Pearmain.		
1st Phase 22 days.	2nd Phase 72 days.	3rd Phase 56 days.	1st Phase 22 days.	2nd Phase 55 days.	3rd Phase (part only) 15 days.
No starch	Starch increasing	Starch hydrolysing			

*Actual Increase in Dry Matter (Gm. per Apple).*

0.672	10.06	6.69		0.530	8.02	3.26
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*Increase in Constituents as Percentage of the Dry Weight Increase per Apple.*

cellulose and hemi- cellulose	38.9	15.4	9.0		44.4	18.5	11.3
acid	15.8	11.5	5.3		10.1	5.6	2.7
acid + cellulose and hemicellulose	54.7	26.9	14.3		54.5	24.1	14.0
starch	none	12.8	-19.2		none	13.4	-4.3
fructose	5.8	25.3	47.6		6.0	33.7	50.0
total sugar	15.7	55.5	95.3		15.8	58.4	88.0
sugar less starch hydrolysed.	—	—	76.1		—	—	83.7
unaccounted for	29.6	4.8	9.6		29.7	4.1	2.3

Although fructose constitutes about 50 per cent. of the dry material stored during starch hydrolysis in both varieties, the amount is slightly greater in Worcester Pearmain, while storage of sucrose is favoured in the more acid Bramley's Seedling. The accumulation of rather more sucrose in these apples seems to be associated with formation of a slightly higher percentage of the unestimated substances. The chief differences in the storage of material in the two varieties are therefore in the amounts of fructose and acid accumulated.

## PART II. THE EFFECT OF TIME OF GATHERING ON THE CHEMICAL CHANGES IN APPLES STORED AT 1° C.

The data showing the chemical changes occurring in the final phase of the ripening of the apple on the tree show that in the slow-ripening varieties like Bramley's Seedling, which are not gathered until almost free from starch, the changes in concentration during the last three weeks on the tree (i.e. prior to commercial gathering date) are very small, although

growth may be still continuing at a constant rate. The observation (40) that crude chemical composition is very little guide to the degree of maturity at gathering is thus confirmed. In sub-acid varieties, such as Worcester Pearmain, there is, however, a considerable change of sugar concentration in the weeks prior to the commercial gathering date, although the concentration of total solids remains practically constant. This may be accounted for by the fact that these apples are picked at a much earlier stage physiologically than the Bramley's Seedling, and still contain up to 1 per cent. of starch (wet weight basis).

The amount of starch present and the ratio of sugar concentration to total solid concentration would seem to be the best guide to maturity to be obtained from chemical analysis. The maximum value for the ratio at the time of starch disappearance is 70 per cent. in both varieties.

Although concentration changes are small in the final stage of ripening, nevertheless changes do occur which affect the subsequent behaviour in store. Thus Haynes and Archbold (see 23) found that an early-gathered sample of Bramley's Seedling apples was less susceptible to internal breakdown in cold store than one gathered a month later, although the mean rate of loss of solids in respiration was greater in the early-gathered set. The present investigation of the chemical changes taking place in fruit gathered at different times has been carried out to determine the differences in the rates of change of the carbohydrates during storage at 1° C., brought about by variation in the gathering date. Changes may be due to differences in enzyme activity, physical state (size, softness, &c.), or the state of the protoplasm (34), all of which may affect the oxidatory processes. It is well known that respiration is a slow process in the apple, and only a small part of the sugar present is lost during a prolonged storage period at 1° C. Differences in the nature of the sugars oxidized may therefore be important, as well as differences in the total rate of loss of sugar.

#### *Methods and Material for Analysis.*

The methods of analysis used were those described in Part I. Four pickings were made of Bramley's Seedling during the 1929 season, one at the commercial gathering date (October 10), two before this date (September 9 and 21), and one later (October 22). Two pickings of Worcester Pearmain were also made, one at the commercial gathering date (September 17), and one three weeks earlier (August 26). The fruit was gathered from the same trees as the samples used for the study of changes during growth (see p. 409). At each date about 300 apples were gathered and sent by train to the cold store, arriving on the day following picking. Each apple was then weighed, wrapped in an oiled wrapper, and the whole picking placed in store at 1° C., and about 85 per cent. humidity.

Samples of twenty apples from each picking were then withdrawn from store at frequent intervals (7-14 days) over a period of about nine months, and analysed. The results, expressed as percentage of original fresh weight at time of gathering (see 32, p. 983), obtained for the four pickings of Bramley's Seedling and two of Worcester Pearmain are shown in Tables VIII to XIII. The original fresh weight is denoted as O.W. in all tables and figures. The amounts of sugar, acid, and alcohol-insoluble material estimated account for all but 1-1.5 per cent. of the wet weight or 7-8 per cent. of the dry weight of the apple. Estimates of sugar content made from the density of expressed juice have been shown to be higher than those obtained by direct estimation (3), suggesting that the material not accounted for was a soluble, non-reducing substance of a carbohydrate nature.

Although the sum of these estimated constituents is always less than the total dry material, it has been found that the sum of the losses of sugar, acid, and alcohol-insoluble material is always slightly greater than the loss of dry material, so that in addition to a loss of carbon in respiration there appears to be a small conversion of one of these three substances to a soluble non-reducing material not estimated (see p. 451).

The initial composition and average weight per apple of these six sets of apples when gathered may be seen in Tables I and IV, where the analyses of Worcester Pearmain for 26. 8. 29 and 17. 9. 29, and of Bramley's Seedling for 10. 9. 29, 26. 9. 29, 10. 10. 29, and 22. 10. 29, represent the composition at the six gathering dates. The nitrogen concentration is a little lower in the two sets of Worcester Pearmain than in the four sets of Bramley's Seedling, but is not affected in either case by the gathering date. In Worcester Pearmain the percentage dry weight was the same in the two pickings, but sugar content was higher in the second picking, and starch was present at gathering in both cases. In Bramley's Seedling the dry weight of the 2nd, 3rd, and 4th pickings is nearly the same, and that of the 1st picking rather lower than the other three. Sugar content increased from the 1st to the 4th picking, and starch was present at gathering in the 1st and 2nd pickings, but only traces remained when the other two sets were gathered.

The changes in the various constituents of the 1st picking of Worcester Pearmain and the 3rd picking of Bramley's Seedling during a period of 7-9 months in store at 1° C. are shown in Figs. 6 and 7. The Worcester Pearmain set illustrates the case when starch is present at gathering, and the Bramley's Seedling the case when starch hydrolysis is complete at the gathering date. The changes in the sugars of the 2nd picking of Worcester Pearmain and the 1st, 2nd, and 4th pickings of Bramley's Seedling are shown in Figs. 8, 9, and 10.

TABLE VIII.  
*Analyses of Brandley's Seedling Apples during Storage at 1° C. Gathered and put into Store September 10, 1929.*  
*1st Picking.*

Results expressed as percentage of fresh weight at time of gathering. Mean nitrogen, 0.0262 per cent.

Date of Analysis.	Days of storage.	Loss of wt. of apple.	Dry wt.	St + R + A.	Total Sugar.	Reducing Sugar as F + G.	Sucrose as $C_{12}H_{22}O_{11}$ .	Fructose.	Glucose.	Alcohol-insol. residue (including Starch).	Acid as Malic.	Starch.
		%	W		St	Sr	Ss	F	G	R	A	
10.9.29	1	0.00	13.77	12.75	8.51	6.93	1.58	4.98	1.95	3.12	1.240	0.70
16.9.29	7	0.23	13.60	12.53	8.53	6.79	1.74	4.96	1.83	2.75	1.249	0.43
23.9.29	14	0.54	13.55	12.39	8.59	6.90	1.69	4.92	1.98	2.61	1.187	0.32
30.9.29	21	1.11	13.49	12.51	9.03	7.11	1.92	4.99	2.12	2.27	1.210	0.08
7.10.29	29	1.10	13.37	12.30	9.03	7.13	1.90	4.93	2.20	2.13	1.142	0.07
21.10.29	43	1.58	13.11	12.37	9.21	7.58	1.63	5.31	2.27	2.06	1.099	0.02
4.11.29	57	2.11	13.59	12.28	9.09	7.61	1.48	5.61	2.00	2.05	1.137	0.00
18.11.29	71	2.33	—	12.43	9.20	7.62	1.58	5.31	2.31	2.12	1.112	—
2.12.29	85	3.47	13.15	11.94	8.88	7.51	1.37	5.62	1.89	2.03	1.030	—
16.12.29	99	3.87	13.24	12.04	9.06	7.73	1.33	5.38	2.35	1.97	1.012	—
6.1.30	120	4.31	13.09	11.72	8.83	7.67	1.16	5.44	2.23	1.91	0.981	—
27.1.30	141	6.22	12.87	11.69	8.74	7.64	1.10	5.45	2.19	2.03	0.924	—
10.2.30	155	6.82	12.21	11.17	8.39	7.50	0.89	5.43	2.07	1.89	0.887	—
24.2.30	169	7.64	12.43	11.25	8.51	7.71	0.80	5.58	2.13	1.88	0.860	—
10.3.30	183	7.74	11.99	11.04	8.38	7.69	0.69	5.52	2.17	1.79	0.865	—
7.4.30	215	9.31	11.79	10.89	8.29	7.48	0.81	5.38	2.10	1.79	0.808	—

TABLE IX.  
*Analyses of Bramley's Seedling Apples during Storage at 1° C. Gathered and put into Store September 26, 1929.  
and Picking.*

Results expressed as percentage of fresh weight at time of gathering. Mean nitrogen, 0.0263 per cent.

Date of Analysis.	Days of storage.	Loss of wt. of apple. %	Dry wt. W	St + R + A.	Total Sugar. St	Reducing Sugar as F + G. Sr	Sucrose as $C_{12}H_{22}O_{11}$ Ss	Fructose. F	Glucose. G	Alcohol-insol. residue (including Starch). R	Acid as Malic. A	Starch.
26. 9. 29	1	0.00	14.20	13.29	9.60	8.08	1.52	5.69	2.39	2.54	1.154	0.37
10. 10. 29	16	0.50	—	13.45	9.81	7.62	2.19	—	—	2.52	1.120	0.15
24. 10. 29	30	0.59	14.28	13.25	10.06	8.09	1.97	5.97	2.12	2.12	1.066	0.03
29. 10. 29	35	0.87	14.43	13.38	10.03	8.10	1.93	6.03	2.07	2.20	1.149	0.05
6. 11. 29	43	1.05	14.28	13.33	9.99	8.15	1.84	6.29	2.18	2.19	1.149	0.00
20. 11. 29	57	1.46	14.54	13.41	10.16	8.43	1.73	6.46	1.97	2.17	1.076	—
4. 12. 29	71	2.42	13.99	13.05	9.89	8.10	1.79	6.03	1.97	2.09	1.065	—
18. 12. 29	85	3.26	13.80	12.73	9.73	8.19	1.54	6.05	2.14	2.01	0.992	—
9. 1. 30	107	3.30	13.42	12.22	9.28	8.23	1.05	5.89	2.34	1.96	0.984	—
30. 1. 30	128	3.82	13.56	12.52	9.65	8.33	1.32	6.06	2.27	2.00	0.865	—
12. 2. 30	141	3.86	13.52	12.09	9.30	8.20	1.10	5.95	2.25	1.91	0.884	—
26. 2. 30	155	4.45	13.36	12.10	9.38	8.40	0.98	6.07	2.33	1.86	0.859	—
12. 3. 30	169	5.34	13.06	11.67	8.90	7.94	0.96	—	—	1.87	0.897	—
25. 3. 30	182	5.59	12.84	11.62	8.93	8.11	0.82	5.84	2.27	1.87	0.823	—
28. 4. 30	216	7.10	12.45	11.16	8.60	7.85	0.75	5.60	2.25	1.78	0.789	—
21. 7. 30	300	12.83	11.74	10.37	7.99	7.48	0.51	5.49	1.99	1.76	0.615	—

TABLE X.

*Analyses of Bramley's Seedling Apples during Storage at 1° C. Gathered and put into Store October 10, 1929.*

*3rd Picking.*

Results expressed as percentage of fresh weight at time of gathering. Mean nitrogen, 0.0291 per cent.

Date of Analysis.	Days of storage.	Loss of wt. of apple. %	Dry wt. W	St + R + A.	Total Sugar. St	Reducing Sugar as F + G. Sr	Sucrose as $C_{12}H_{22}O_{11}$ . Ss	Fructose. F	Glucose. G	Alcohol-insol. residue (including Starch). R	Acid as Malic A	Starch.
10. 10. 29	1	0.00	—	—	—	—	—	—	—	—	—	trace
17. 10. 29	8	0.22	14.44	13.71	10.49	8.46	2.03	6.25	2.21	2.09	1.113	none
29. 10. 29	20	0.18	14.53	13.44	10.39	8.46	1.93	6.28	2.18	2.05	0.998	"
5. 11. 29	27	0.41	14.22	13.40	10.22	8.49	1.73	6.16	2.33	2.07	1.114	"
13. 11. 29	35	0.68	14.24	—	—	—	—	—	—	2.02	1.060	"
19. 11. 29	41	0.61	—	—	—	—	—	—	—	2.10	1.021	"
3. 12. 29	55	1.16	14.52	13.68	10.56	8.59	1.97	6.52	2.07	2.07	1.010	"
17. 12. 29	69	1.77	14.12	13.04	10.07	8.76	1.73	6.57	2.19	2.07	0.999	"
31. 12. 29	83	1.96	14.25	13.22	10.27	8.53	1.54	6.38	2.15	1.95	0.976	"
15. 1. 30	98	2.38	13.67	12.49	9.54	8.75	1.52	6.62	2.13	1.97	0.947	"
28. 1. 30	111	3.09	13.65	12.49	9.64	8.22	1.32	5.90	2.32	1.95	0.899	"
11. 2. 30	124	3.51	13.71	12.53	9.69	8.46	1.18	6.14	2.32	1.95	0.830	"
25. 2. 30	138	3.95	13.48	12.24	9.54	8.54	1.15	6.37	2.17	2.01	0.871	"
11. 3. 30	152	4.58	13.69	12.46	9.70	8.56	0.98	6.24	2.32	1.83	0.818	"
25. 3. 30	166	4.99	13.21	11.90	9.16	8.75	0.95	6.49	2.26	1.97	0.817	"
8. 4. 30	180	5.18	13.21	12.01	9.32	8.21	0.95	5.91	2.30	1.92	0.763	"
28. 4. 30	200	6.54	12.96	11.85	9.22	8.47	0.85	6.15	2.32	1.86	0.769	"
12. 5. 30	214	7.40	12.57	11.59	9.05	8.27	0.95	6.00	2.27	1.83	0.714	"
26. 5. 30	228	7.76	12.40	11.19	8.74	8.24	0.81	6.03	2.27	1.76	0.692	"
21. 7. 30	284	10.41	12.02	10.86	8.50	7.80	0.64	5.82	1.98	1.81	0.547	"

TABLE XI.

*Analyses of Bramley's Seedling Apples during Storage at 1° C. Gathered and put into Store October 22, 1929.  
4th Picking.*

Results expressed as percentage of fresh weight at time of gathering. Mean nitrogen, 0.0285 per cent.

Date of Analysis.	Days of storage.	Loss of wt. of apple. %	Dry wt. W	St + R + A.	Total Sugar. St	Reducing Sugar as F + G. Sr	Sucrose as $C_{12}H_{22}O_{11}$ . Ss	Fructose. F	Glucose. G	Alcohol-insol. residue (including Starch). R	Acid as Malic. A	Starch.
22. 10. 29	1	0.00	14.42	13.34	10.15	7.34	2.81	5.73	1.61	2.12	1.066	trace
6. 11. 29	16	0.39	14.38	13.25	9.93	7.33	2.60	5.78	1.55	2.13	1.186	none
13. 11. 29	23	0.89	14.45	13.32	10.23	7.65	2.58	5.72	1.93	2.08	1.006	"
20. 11. 29	30	0.94	—	—	—	—	—	—	—	2.06	1.023	"
26. 11. 29	36	1.24	14.40	13.01	10.09	7.74	2.35	6.12	1.62	1.95	0.973	"
4. 12. 29	44	1.19	14.35	13.28	10.33	8.07	2.26	5.86	2.21	1.96	0.986	"
18. 12. 29	58	2.11	13.94	12.82	9.97	8.14	1.83	6.39	1.75	1.90	0.948	"
31. 12. 29	71	2.59	13.91	12.60	9.83	7.93	1.90	6.22	1.71	1.88	0.887	"
16. 1. 30	87	3.39	14.07	12.78	9.96	8.25	1.71	6.45	1.80	1.92	0.904	"
30. 1. 30	101	3.80	13.74	12.66	9.87	8.22	1.65	6.42	1.80	1.96	0.830	"
12. 2. 30	113	4.23	13.81	12.44	9.76	8.08	1.68	6.25	1.83	1.82	0.856	"
26. 2. 30	127	4.90	13.84	12.50	9.72	8.12	1.60	6.03	2.09	1.92	0.855	"
12. 3. 30	141	5.66	13.66	12.30	9.48	8.04	1.44	5.92	2.12	1.95	0.866	"
25. 3. 30	154	5.90	13.39	12.20	9.43	7.92	1.51	5.87	2.05	1.93	0.836	"
8. 4. 30	168	6.55	13.31	12.12	9.42	7.92	1.50	5.87	2.05	1.84	0.762	"
12. 5. 30	202	7.43	12.78	11.55	8.99	7.85	1.14	5.87	1.98	1.81	0.748	"

TABLE XII.

*Analyses of Worcester Pearmain Apples during Storage at 1° C. Gathered and put into Store August 26, 1920.*

*1st Picking.*

Results expressed as percentage of fresh weight at time of gathering. Mean nitrogen, 0.0230 per cent.

Date of Analysis.	Days of Storage.	Loss of wt. of apple.	Dry wt.	St + R + A.	Total sugar.	Reducing sugar as F + G.	Sucrose as $C_{12}H_{22}O_{11}$ .	Fructose.	Glucose.	Alcohol-insol. residue (including Starch).	Acid as Malic.	Starch.
		%	W		St	Sr	Ss	F	G	R	A	
26. 8. 29	1	0.00	17.15	15.46	10.74	8.77	1.97	6.74	2.03	4.04	0.677	0.96
3. 9. 29	9	1.48	—	15.65	11.33	9.45	1.88	7.40	2.05	3.80	0.519	0.55
10. 9. 29	16	3.20	17.11	15.70	11.71	9.58	2.03	7.63	2.05	3.43	0.559	0.58
16. 9. 29	22	3.24	17.00	15.53	11.82	9.86	1.96	7.72	2.14	3.16	0.531	0.35
23. 9. 29	29	3.80	17.06	15.46	11.86	9.80	2.06	7.83	1.97	3.12	0.475	0.07
7. 10. 29	43	6.62	16.76	15.11	11.96	10.15	1.81	8.11	2.04	2.67	0.477	0.10
21. 10. 29	57	8.13	16.48	14.98	11.94	10.51	1.43	8.33	2.18	2.62	0.415	0.06
4. 11. 29	71	8.62	16.52	14.69	11.64	10.49	1.15	8.12	2.37	2.63	0.420	0.03
18. 11. 29	85	10.13	—	14.95	11.86	11.00	0.86	8.82	2.18	2.69	0.395	0.00
2. 12. 29	99	11.74	15.98	14.04	11.16	10.46	0.70	8.39	2.07	2.53	0.352	"
16. 12. 29	113	16.43	15.33	14.02	11.23	10.52	0.71	8.41	2.11	2.47	0.322	"
6. 1. 30	134	17.62	15.95	13.77	11.12	10.58	0.54	8.55	2.03	2.36	0.292	"
27. 1. 30	155	18.04	15.15	13.52	10.80	10.24	0.56	8.20	2.04	2.45	0.269	"
10. 2. 30	169	20.46	14.87	13.43	10.79	10.34	0.45	8.52	1.82	2.39	0.251	"
10. 3. 30	197	26.00	14.57	12.81	10.30	9.95	0.35	8.23	1.72	2.32	0.193	"



TABLE XIII.

*Analyses of Worcester Parmain Apples during Storage at 1° C. Gathered and put into Store September 17, 1929 and Picking.*

Results expressed as percentage of fresh weight at time of gathering. Mean nitrogen 0.0237 per cent.

Date of Analysis.	Days of Storage.	Loss of wt. of apple. %	Dry wt. W	St + R + A.	Total sugar. St	Reducing sugar as F + G. Sr	Sucrose as $C_{12}H_{22}O_{11}$ . Ss	Fructose. F	Glucose. G	Alcohol-insol. residue (including Starch). R	Acid as Malic. A	Starch.
17.9.29	1	0.00	16.99	15.41	12.15	10.19	1.96	8.24	1.95	2.89	0.371	0.48
23.9.29	7	2.34	16.78	15.34	12.22	10.22	2.00	8.10	2.12	2.72	0.396	0.28
30.9.29	14	2.60	16.65	14.90	11.98	10.09	1.89	8.07	2.02	2.57	0.350	0.22
7.10.29	21	1.94	16.50	14.93	12.12	10.28	1.84	7.93	2.35	2.44	0.374	0.12
21.10.29	35	3.75	16.09	14.41	11.83	10.41	1.42	8.55	1.86	2.25	0.333	0.02
4.11.29	49	4.74	15.82	14.00	11.34	9.86	1.48	8.25	1.61	2.32	0.339	0.04
18.11.29	63	7.55	—	14.34	11.75	10.58	1.17	8.28	2.30	2.27	0.318	0.00
2.12.29	77	9.49	16.22	14.14	11.64	10.36	1.28	8.44	1.92	2.22	0.279	—
16.12.29	91	9.47	15.63	13.96	11.51	10.31	1.20	8.38	1.93	2.17	0.276	—
6.1.30	112	11.00	15.61	13.67	11.41	10.67	0.74	8.83	1.84	2.04	0.223	—
27.1.30	133	14.25	15.45	13.61	11.41	10.74	0.67	8.93	1.81	2.02	0.177	—
10.2.30	147	12.73	15.09	13.35	11.11	10.58	0.53	8.79	1.79	2.06	0.184	—
24.2.30	161	15.00	15.06	13.51	11.22	10.67	0.55	8.83	1.84	2.12	0.165	—
10.3.30	176	17.36	14.89	13.08	10.82	10.32	0.50	8.26	2.06	2.07	0.187	—
7.4.30	204	18.14	15.10	13.34	11.14	10.69	0.45	8.81	1.88	2.05	0.149	—
21.7.30	308	22.81	13.89	12.19	10.14	9.73	0.41	8.03	1.70	1.96	0.090	—

The starch content at gathering of the 1st picking of Worcester Pearmain (26.8.29) was 0.96 per cent., and of the 1st picking of Bramley's Seedling (9.9.29) was 0.69 per cent. Premature gathering and storage at 1°C. had little effect on the rate of starch hydrolysis, which proceeded at a decreasing rate both on the tree and in storage, and was completed at the

same time in each case.

Only small traces of starch remained in the 1st picking of Worcester Pearmain after 43 days, and of Bramley's Seedling after 30 days. By plotting the data for starch given in Tables VIII to XIII it may be seen that starch hydrolysis follows an exponential law very closely. In both varieties starch hydrolysis was accompanied by a loss of other insoluble material. Widdowson (66) has shown that two hemicelluloses, one a true polysaccharide and one a polyuronide, can be extracted from the apple by N/75 HCl, and that it is one or both of these which decrease in amount during storage. The amount of insoluble material other than starch in the first picking of Worcester Pearmain is shown in Fig. 6.

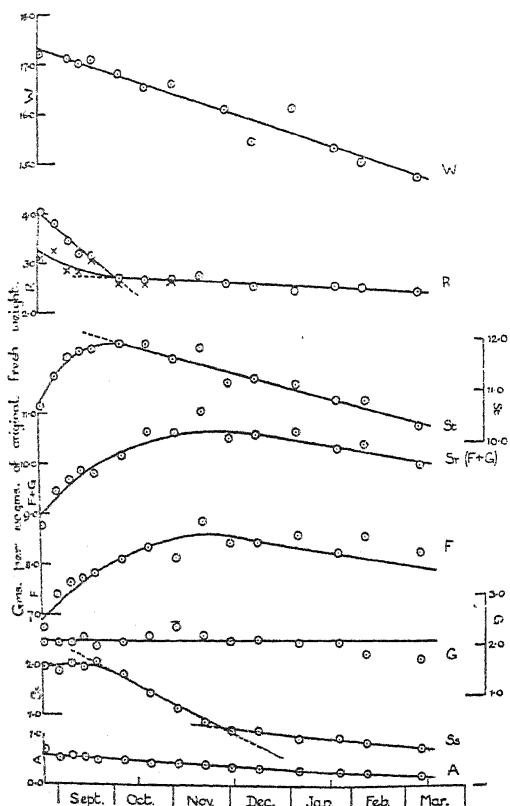


FIG. 6. Quantity-time curves for the constituents of Worcester Pearmain apples during storage at 1°C. (1st picking gathered 26.8.29). Dry weight, W, alcohol-insoluble material including starch when present, R (crosses show insoluble material other than starch). Total sugar, St, reducing sugars, Sr, fructose, F, glucose, G, sucrose, Ss, acid, A. Dotted lines are continuations of calculated lines of closest fit.

The total sugar content rises at first, reaching a maximum value as starch disappears (see Figs. 6 to 9). In the Worcester Pearmain the increase in sugar content was equal to the whole loss of alcohol-insoluble material (including starch) for the first 30 days in store, suggesting that sugar consumption does not begin until nearly all the starch has gone, and that for a short time only acid was being oxidized. In Bramley's Seedling the increase of sugar was equal to the loss of starch, so that both acid and either the products of hydrolysis of hemicellulose

only, or of starch and some hemicellulose, were lost. These points, however, need further study.

In the second pickings the initial amounts of starch present were 0.4 per cent. in Worcester Pearmain and 0.37 per cent. in Bramley's

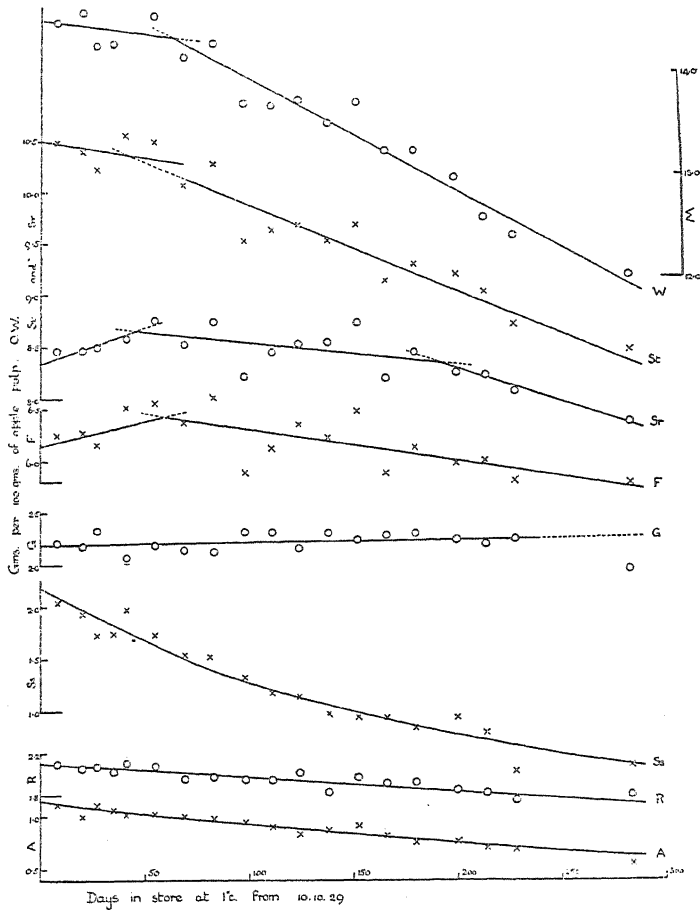


FIG. 7. Quantity-time curves for the constituents of Bramley's Seedling apples during storage at 1°C. (3rd picking gathered 10.10.29). Constituents denoted by same letters as in Fig. 6. Dotted lines are continuation of calculated lines of closest fit.

Seedling, and only traces remained after 21 and 18 days, respectively, in cold storage. There was no increase in sugar content of Worcester Pearmain, so that sugar oxidation at this stage was greater than the rate of starch hydrolysis; but in Bramley's Seedling there was a small increase (see Figs. 8 and 9).

In Worcester Pearmain the sugar produced during starch hydrolysis was fructose, and neither during the last three weeks on the tree (see p. 422),



at rather long intervals of storage life suggested that the rate of sugar loss might decrease slightly, and this seems possibly the case also in the two pickings of Worcester Pearmain. In these earlier results (32) curves

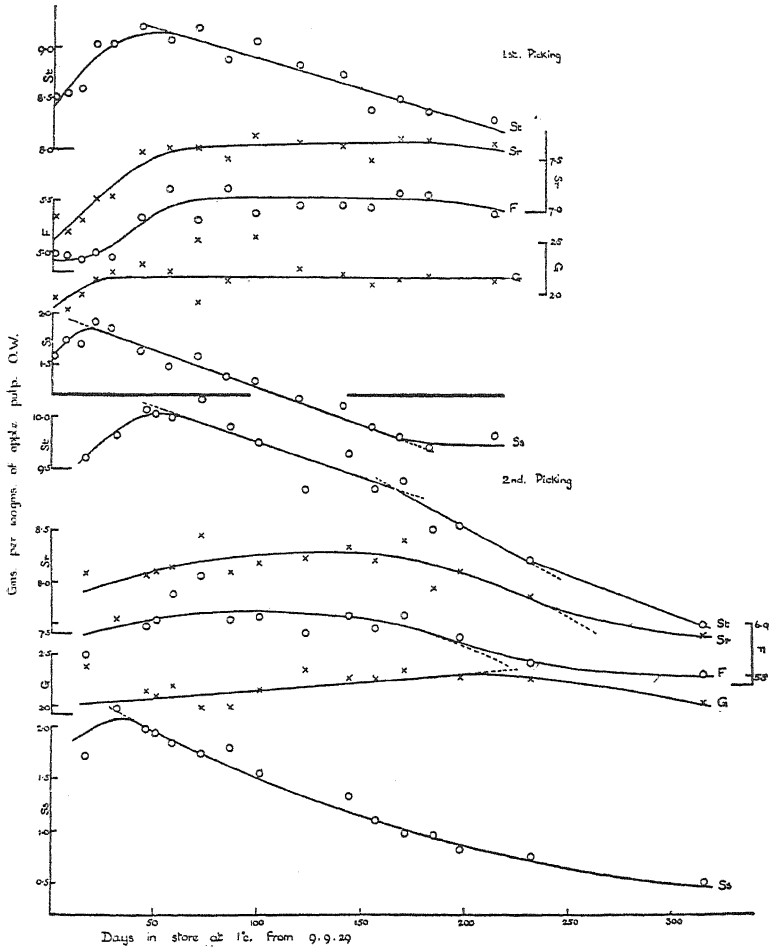


FIG. 9. Quantity-time curves for total sugar, St, reducing sugars, SR, fructose, F, glucose, G, and sucrose, Ss, in the 1st and 2nd pickings of Bramley's Seedling apples during storage at 1°C. (gathering dates 10.9.29 and 21.9.29).

were fitted to the experimental data in order to calculate the rates of loss of the various constituents. It was found, however, that more frequent analyses were necessary if inflexions in the curves, indicating changes in rate, were to be defined. In the present investigation, the samples were chosen so that the significance of the differences in rate of loss between two sets of apples, and of changes in rate in the same set of apples could be measured. For this purpose all the pickings were made entirely at

random, and there was no selection for size, position on tree, or any other reason. This procedure has, of course, produced rather a large scatter in the results, but it was thought important to ensure that the data were

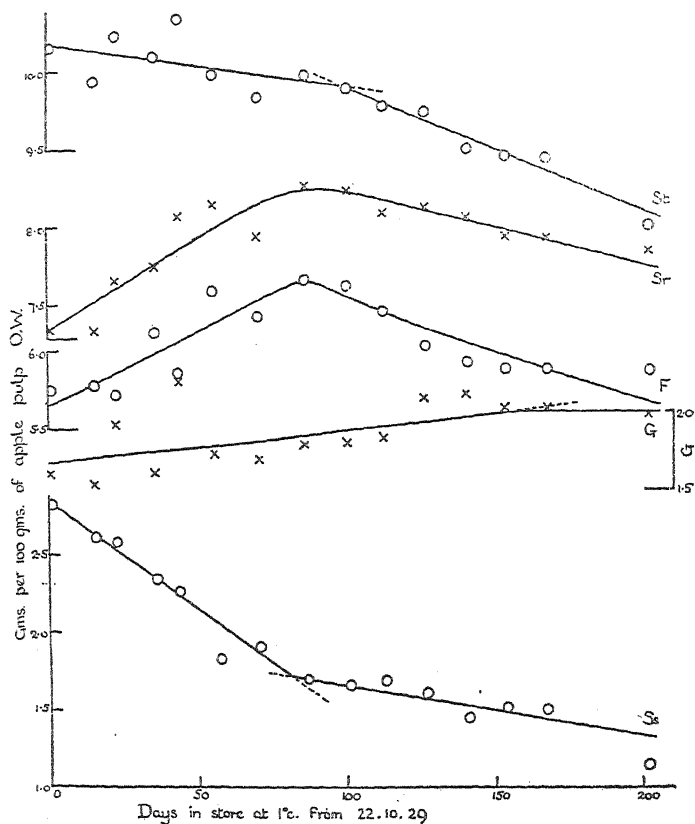


FIG. 10. Quantity-time curves for total sugar, ST, reducing sugars, Sr, fructose, F, glucose, G, and sucrose, Ss, in the 4th picking of Bramley's Seedling apples during storage at 1° C. (gathering date 22.10.29).

entirely suitable for statistical treatment, and that any significant result obtained should apply to the whole population of apples and not to some selected fraction only.

To obtain a measure of significance of the differences in the calculated rates of loss, the number of apples in the samples from the 3rd picking (normal gathering date October 10) was reduced to 10, and each apple was analysed separately, giving 10 results for each date throughout the storage life, a total of 150 observations. The measures of significance obtained from the results of the analyses of the 3rd picking have been applied to the other sets where only analysis of mixed samples was possible. Since

these samples consisted of 20 apples each, and since it is known that the 'scatter' tends to be smaller in early pickings (23), this seems to be justifiable. The means of the values obtained for the 10 apples of the 3rd picking are shown in Table X.<sup>1</sup>

The data representing total sugar content of the 3rd picking of Bramley's Seedling (see Fig. 7) during storage suggest that the rate of loss increases after about 8 weeks in store. It is difficult to define at all precisely the points of inflexion in a curve fitted to data of this type, since the mean values plotted will necessarily tend to smooth out any inflexions. In order to test whether this apparent change of rate was significant, regression lines were calculated for the observations up to the 69th day in store, and for those from the 55th-228th day in store. These lines are shown in Fig. 7. From the equations to these straight lines it was found that the mean rate of loss of sugar up to day 69 was 0.00355 gm. per 100 gm. (fresh weight at time of gathering) per day, and from day 55 to 228 0.0081 gm. per 100 gm. per day. The significance of the difference between these two estimates of the rate of loss of sugar was then tested by methods recommended by Fisher (22), using the results of the analyses of single apples. It was found that differences from the value 0.0081 greater than 0.0021 were significant, and that the value 0.00355 was not significantly different from zero. Since in all other pickings a small apparent loss of total sugar also occurs in the first weeks of storage (after starch has disappeared), it must be concluded that there is a small consumption of sugar (although it cannot be demonstrated with certainty by the data given here) during the first 69 days in store in the 3rd picking. It is also concluded that a significant increase in the rate of loss occurs after this time.

At the time that the rate of total sugar consumption begins to increase (after 69 days) the maximum value of reducing sugars occurs. The rates of loss of total sugar from the time of starch disappearance up to the time of the reducing sugar maximum and for the time subsequent to this were, therefore, compared in the other sets of apples. The results are shown in Table XIV. The limit of significance for the differences will depend on the number of observations used in calculating the rates. The differences which can be regarded as significant are marked with an asterisk in the Table. In the 1st picking of Bramley's Seedling there is an apparent increase in the rate of loss of sugar after about a 100 days in store, when the reducing sugars have reached a value which remains practically constant for some time (see Fig. 9), but it cannot be shown to be significant. The other three

<sup>1</sup> Publication of the complete data obtained by the analysis of single apples would make the paper unduly bulky. Consequently a copy of the data has been deposited in the Zoological Library of the British Museum (Natural History), and both this and the author's copy at the Imperial College of Science are available for consultation.

pickings all show a significant increase in the rate of loss of total sugar as the maximum value of reducing sugars is reached. From Table XIV it is seen that the rate of loss of total sugar while reducing sugars are increasing is very low in late-gathered fruit.

TABLE XIV.

*Rates of Loss of Total Sugar in Bramley's Seedling and Worcester Pearmain Gathered at Different Dates and Stored at 1° C.*

*a.* while reducing sugars are increasing, and after starch disappearance.

*b.* while reducing sugars are decreasing.

	Period in store. Days.		Rate of loss of sugar gm. per 100 gm. (O.W.) per day.		Difference.
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>b-a</i>
Worcester Pearmain, 1st picking	43-113	113-197	0.01226	0.01097	-0.00129
2nd picking	1-112 <sup>1</sup>	112-204	0.00704 <sup>1</sup>	0.00446	-0.00258 *
Bramley's Seedling, 1st picking	reducing sugars rise throughout the storage period				
2nd picking	30-128	128-216	0.00656	0.01100	0.00454 *
3rd picking	8-69	55-228	0.00334	0.00819	0.00485 *
4th picking	1-101	87-202	0.00288	0.00786	0.00498 *

In Worcester Pearmain there is no evidence of an increase in the rate of loss of sugar during storage; in both pickings the rate of loss was lower in the second half of storage life, and in the 2nd picking the fall in rate as starch disappeared was significant (see Figs. 6 and 8).

The average rates of loss of sugar during the whole storage period (after starch has gone) are shown in Table XVII, together with the average rates of loss of the other constituents. In Bramley's Seedling the rate of loss is highest in the 2nd picking and decreases to the 4th, although the initial concentration of sugar increases, while the rate is also low in the 1st picking. The values for the 2nd and 3rd pickings are significantly different from those for the 1st and 4th, while the values in Table XIV for the rates of loss of the 3rd and 4th pickings are significantly different from those of the 2nd. The rate of loss of sugar thus has a maximum value in the 2nd picking. The time at which this maximum was found was a fortnight before the normal gathering date. In Figs. 6-10 the change of sugar-content has been represented by two straight lines where the change in rate of loss can be shown to be significant, and in the other cases by one straight line through all the observations. These lines are not intended to be an exact representation of the course of sugar change, but a means of determining the average rate of loss over a given period. Actually the rate of loss probably increases continuously (see p. 454).

<sup>1</sup> This value includes the period of starch hydrolysis.



*Glucose, Fructose, and Sucrose.*

In the 1st picking of Worcester Pearmain, glucose content remained constant for 160 days, but the last two observations made after 169 and 197 days, respectively (see Fig. 6), suggested that there may be a slight loss of glucose about this time. In the 2nd picking there appeared to be a fall throughout storage life, but the scatter of results in this set was large, and the fall could not be shown to be significant (see Fig. 8). Glucose content thus remains constant, or may fall slightly in Worcester Pearmain apples stored at 1° C.

In Bramley's Seedling, in the 1st picking, the glucose content increased during starch hydrolysis, and then remained constant throughout the rest of the storage period. In the 2nd, 3rd, and 4th pickings the glucose content increased slowly for about 200 days, the increase being most marked in the 4th picking. One sample of the 2nd picking was kept for analysis after 300 days, and the amount of glucose had apparently decreased a little by this time. In Bramley's Seedling, therefore, glucose content remains constant, or increases a little for about 200 days, after which it may fall slowly (see Figs. 7, 9, and 10).

The rate of sucrose inversion in all the sets proceeded either at a continuously decreasing rate (cf. Fig. 9), or else the rate of loss was nearly constant for some time and then diminished rather suddenly (cf. Fig. 10). Inversion practically ceased when some sucrose still remained. In the first months in store the rate of sucrose inversion exceeds that of sugar oxidation and there is a rise of reducing sugars, which is all or nearly all due to an increase of fructose. In some cases (3rd and 4th pickings of Bramley's Seedling, and 2nd picking of Worcester Pearmain) the initial rate of inversion is more than double the rate of sugar loss (see Tables XIV and XV), but still only fructose is accumulated. If the glucose produced by inversion is preferentially oxidized, the excess of this sugar produced from the sucrose and not respired must be rapidly converted to fructose. As the rate of inversion falls and the rate of loss of total sugar tends to increase, fructose is apparently respired in addition to glucose and, when the maximum value of reducing sugar is reached, equal amounts of the two sugars will be consumed, and rate of inversion will equal rate of consumption. Reducing sugars then begin to fall, and the sugar lost is fructose. The increased rate of sugar loss already noted occurs at this time. It seems likely from the final observation of the 2nd picking, after 300 days' storage, that the rate of sugar loss has declined again after this time.

It will be seen from the figures that the time taken to reach the maximum reducing sugar content and the form of the curves representing sucrose content are markedly affected by the gathering date. Thus, in the 1st picking of Bramley's Seedling, reducing sugars increased fast at first,

owing to starch hydrolysis, and then very slowly for a prolonged period (see Fig. 9). The final observation after 215 days' storage showed a low value, so that it is probable that reducing sugar begins to fall about this time. In the 2nd picking, where the starch content was low at gathering, there was a slow rise to the maximum value, which was reached in 125 days, and in the following 40 days only 0.04 grm. of reducing sugar was lost; the rate of fall then increased markedly.

In the 3rd picking the maximum value was reached in 70 days (see note on p. 447) and was followed first by a long period of slow rate of loss, and then, after 150 days, by a period of increased rate of loss. In the 4th picking the maximum value was reached in 80 days and fructose diminished rather rapidly at once, the period of slow rate of change of reducing sugars being absent. The results of the 4th picking suggest that some fructose is being converted to glucose in the second part of storage life, since it decreases in amount more quickly than the total reducing sugar, and the glucose is rising (see Fig. 10).

It was suggested by Haynes and Archbold (32) that the sucrose content of apples declined logarithmically during the greater part of storage life, but that at the end the rate of loss decreased more quickly than the exponential law requires, and a minimum value of sucrose was reached when inversion almost ceased. Kidd and West (23) found the initial rate of inversion too small for the hydrolysis to be simply exponential, and they also found a 'basal concentration' of sucrose at which inversion ceased. In the present investigation one sample of the 2nd pickings of each variety was kept until July 1930 in order to observe the change in sucrose after a prolonged period (see Tables IX and XIII, and Figs. 8 and 9). In both cases the sucrose content had decreased, so that it must be concluded that sucrose inversion is reduced to a very low value after 5-7 months in storage at 1° C, but that it does still proceed.

In Fig. 9 the curve for sucrose of Bramley's Seedling, 2nd picking, is the exponential curve of closest fit, and it is clear that in this case the loss follows the exponential law very closely, the rate of inversion thus decreasing continuously. Exponential curves are also drawn through the observations for the 3rd picking of Bramley's Seedling and the 2nd picking of Worcester Pearmain. The fit in these cases is not so good, the curve being too steep at the beginning in Bramley's Seedling and too flat in Worcester Pearmain (Figs. 7 and 8) and too steep in both cases at the end of the storage period. The inclusion in the calculation of samples in which, on the one hand, the majority of apples have not yet reached the stage of rapid sucrose inversion, which begins at or towards the end of starch hydrolysis, and, on the other hand, those which have reached the stage of very slow rate of inversion, may account for these discrepancies in the fit of the exponential curve. In the 4th picking of Bramley's Seedling and the 1st

picking of Worcester Pearmain the rate of inversion is rapid and nearly constant at first and diminishes rather abruptly as the reducing sugar maximum is reached. In neither case could the change be expressed by an exponential curve. The change in these two sets has been represented

TABLE XV.

*The Initial Concentration, Initial Rate of Loss, Mean Rates of Loss during the first 100 days in Store, and the 'Minimum' Concentration of Sucrose, and the time from Gathering to the Maximum of Reducing Sugar in Bramley's Seedling and Worcester Pearmain Apples gathered at different dates and stored at 1° C.*

		Sucrose.				
	Days to reducing sugar maximum.	Initial conc. at gather- ing.	Initial rate of loss.	Mean rate of loss, 1st 100 days.	Rate of loss per unit initial conc.	Approx. 'Minimum' conc. grm. per 100 grm. O.W.
		Grm./100 grm. O.W./day.				
Worcester Pearmain—						
1st picking	90	2.14	0.01854	0.01854	0.00960	0.67
2nd picking	140	2.00	0.01622	0.00978	0.00811	0.70
Bramley's Seedling—						
1st picking	183	2.00	0.01184	0.00702	0.00592	0.76
2nd picking	125	2.19	0.01142	0.01012	0.00517	0.80 *
3rd picking	70	2.18	0.01116	0.00870	0.00512	1.00 *
4th picking	75	2.80	0.01402	0.01402	0.00500	1.50

\* Concentrations when reducing sugars begin to fall.

NOTE.—In the 3rd picking of Bramley's Seedling two days elapsed between gathering the fruit and placing it in cold storage, owing to an unfortunate delay in transport. This seems to have caused a rather rapid inversion of sucrose and may account for the rather low initial concentration of this sugar, and for the short time taken to reach the reducing sugar maximum. There was a long period of slow rate of loss of reducing sugar in this picking after the maximum was reached. (See p. 446).

by two straight lines of closest fit calculated for the observations up to the reducing sugar maximum and subsequent to this time. In the 1st picking of Bramley's Seedling the rate of loss diminished slightly (after starch had gone) but not sufficiently for the change to be represented by an exponential curve; it has therefore been represented by a straight line through all the observations. The rate of loss of sucrose is thus markedly affected by gathering date.

The average rate of loss of sucrose for the first 100 days in store, the initial concentration and initial rate of inversion, together with the time taken to reach the reducing sugar maximum are shown in Table XV.

TABLE XVI.

*Mean Rates of Loss or Gain of Reducing Sugars, Fructose, and Glucose in Bramley's Seedling and Worcester Pearmain Apples Gathered on Different Dates and Stored at 1° C.*

*a.* reducing sugars increasing.

*b.* reducing sugars decreasing.

	Reducing sugar. gram. per 100 gram. O.W.		Fructose. per day.		Glucose whole period.
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a + b</i>
Worcester Pearmain—					
1st picking	+0.00828	-0.00629	as reducing sugar		{ no change or
2nd picking	+0.00366	-0.00656	,, ,,		{ slight fall
Bramley's Seedling—					
1st picking		+0.0011	+0.0012		-0.00013 *
2nd picking	+0.0021	-0.0059	+0.0005	-0.0042	+0.00167
3rd picking	+0.0062	-0.0031	+0.0057	-0.0026	+0.00045
4th picking	+0.0114	-0.0048	+0.0092	-0.0068	+0.00203

It will be seen that the rate of loss of sucrose is considerably higher in Worcester Pearmain than in Bramley's Seedling, although there is not much difference in the initial concentrations. The rate of inversion rises in Bramley's Seedling from the 1st to the 4th picking as concentration rises, but in Worcester Pearmain, where there is no difference in concentration, the rate of inversion is lower in the 2nd picking. These results suggest that the rate of inversion per unit concentration decreases with age. The high values for Worcester Pearmain, may be due in part to the fact that these apples were gathered at a physiologically earlier stage than the Bramley's Seedling. The concentration at which inversion nearly ceases is higher in the later pickings. The reduction of the rate of inversion is less abrupt in the 2nd and 3rd pickings, so that the values at the time reducing sugars begin to fall rapidly have been used for comparison (see Table XV). Since the high values of the 'minimum' concentration are associated with a high inversion rate the consumption of reducing sugars will begin after a shorter time in cold store in the later pickings than in the earlier ones. Kidd and West (see 23) have associated a high value of this 'minimum' concentration with bad keeping properties; it is here associated with late gathering.

The curves for reducing sugars in Figs. 6 to 10 have been drawn through the values obtained by subtracting the calculated values of sucrose from those of total sugar. Rates of loss or gain of reducing sugars have also been obtained by difference. The limit of significance for these rates of change of reducing sugars was found by calculation from the observations for the 3rd picking of Bramley's Seedling. In this set

\* Mean rate of loss after starch hydrolysis is complete.

reducing sugars rise at first, reaching a maximum value after about 60 days in store; they then fall slowly until about the 170th day, when the rate of loss increases markedly. Straight lines of closest fit were calculated for the observations from the 55th to the 284th day and for those from the 180th to the 284th day. The mean rates of loss of reducing sugar for these two periods were 0.0019 and 0.00625 grm. per 100 grm. per day. The smallest significant difference for such rates was 0.0011 grm. per 100 grm. per day. Hence there is a definite fall in reducing sugar between the 55th and 166th day, and a definite increase in the rate of loss during the period from the 180th to the 284th day (see Fig. 7).

Straight lines were also calculated to fit the observations of glucose content in each case, and the calculated rates of gain of glucose, together with those of reducing sugars derived as stated above, and of fructose, found as the difference between glucose and reducing sugars, are shown in Table XVI.

The rate of increase of glucose is seen to be highest in the 4th picking. The limit of significance was found to be 0.0006 grm. per 100 grm. per day, so that the rate of increase is real in the 2nd and 4th pickings. It is therefore concluded that glucose-content either remains constant or increases slowly, and that the later pickings are associated with a greater rate of accumulation. In the 4th picking the high rate of inversion, coupled with the low rate of oxidation, results in a rapid rate of accumulation of fructose, and this is followed by a fast rate of consumption when the rate of sucrose inversion is reduced. Late gathering is thus associated with much more marked changes in reducing sugars than early gathering. It has already been pointed out that in the 4th picking there is evidence of a conversion of some of the fructose to glucose.

The changes in sugar content in storage at 1° C. may be briefly summarized as follows. In the first weeks of store sugar is consumed at a slow rate, and reducing sugars, chiefly fructose, rise while sucrose is inverted. The rate of sucrose inversion is greater and of sugar consumption less in later pickings, so that fructose accumulation takes place at a greater rate in these pickings. The initial rate of sucrose inversion per unit concentration falls slightly from the 1st to the 4th picking. The reducing sugars rise rapidly at first and then more slowly, reaching a maximum at times depending on the relative rates of sugar oxidation and of inversion of sucrose. In the earlier pickings there is a prolonged period when reducing sugar hardly changes, but in the final picking of Bramley's Seedling this period was very short. After about five or six months in cold store there is a fall in fructose, and in the later pickings there is some evidence of conversion of fructose to glucose. Glucose remains constant or increases slightly in amount during storage in Bramley's Seedling, but in Worcester Pearmain it may fall a little.

*Acid.*

It has already been shown that the rate of acid consumption in store declines (31, 32); consequently the change in acid has either been represented as an exponential curve or the average rate of loss has been calculated where the change in rate is very small. It has been repeatedly observed that acid consumption increases with the onset of internal breakdown (31, 32). No evidence of this condition was found here, but slight core flush appeared in the 4th picking at the end of the storage period. It may be pointed out that in the cases previously observed (23) the onset of physiological disease occurred when the consumption of stored reducing sugars began, and the rate of inversion of sucrose was much reduced. From the present observations it appears that, although susceptible fruit may break down at this stage of senescence, breakdown does not always occur when sucrose inversion fails to supply sufficient sugar for oxidation. If there is no breakdown the increased consumption of acid is not observed (see Fig. 7, and Tables VIII-XI).

*Alcohol-insoluble Material.*

A small amount of alcohol-insoluble material is lost throughout storage life in all the sets of apples. The rate of loss is a little higher in Worcester Pearmain than in Bramley's Seedling. The average rates of loss, together with the average rates of loss of acid and sugar, and the sum of the rates of loss of acid and sugar, and of acid, sugar, and alcohol-insoluble material, and the rate of loss of total dry weight, are shown in Table XVII. (The period of starch hydrolysis is not included in the calculations for Bramley's Seedling.)

TABLE XVII.

*Mean Rates of Loss of the Constituents of Bramley's Seedling and Worcester Pearmain Apples Gathered at Different Dates and Stored at 1° C.*

	Acid.	Alcohol-insol. material.	Total sugar.				Dry Weight.	Ratio of sugar lost to acid lost.
	A	R	St	St + A	St + A + R	W		
	gram. per 100 gram. O.W. per day.							
Worcester Pearmain—								
1st picking	0.00203	0.00245 *	0.01026 *	0.01229	0.01474	0.01388	5.05	
2nd picking	0.00127	0.00228 *	0.00612	0.00739	0.00961	0.00957	4.82	
Bramley's Seedling—								
1st picking	0.00208	0.00181 *	0.00599 *	0.00807	0.00988	0.00792 *	3.85	
2nd picking	0.00189	0.00236 *	0.00790 *	0.00979	0.01215	0.01055 *	4.18	
3rd picking	0.00195	0.00122 *	0.00765	0.00860	0.00982	0.00893	3.92	
4th picking	0.00145	0.00128 *	0.00526	0.00705	0.00833	0.00767	3.86	

\* Mean rates of loss after starch disappearance.

In Worcester Pearmain, in spite of the low concentration of acid, the rate of acid loss in the 1st picking is equal to that in Bramley's Seedling, and in the 2nd picking is only a little lower. The rate of acid loss per unit concentration is thus much higher in Worcester Pearmain than in Bramley's Seedling. Nevertheless the ratio of sugar to acid lost is high in Worcester Pearmain, owing to the high rate of sugar consumption in this variety. The ratios, which are nearly the same in each picking of the two varieties, are shown in Table XVII.

The rate of loss of alcohol-insoluble material tends to be lowest in the late pickings. The rate of loss of dry material is slightly higher than the sum of the losses of sugar and acid in all cases except the 1st picking of Bramley's Seedling, where it is equal to this sum. It is, however, lower than the sum of sugar, acid, and insoluble material. In Bramley's Seedling the difference from the sum of sugar and acid is very small, and it must be concluded that the amount of material oxidized other than sugar and acid, is almost negligible. In Worcester Pearmain rather a larger proportion of the insoluble material hydrolysed is lost, since the sum of the insoluble material, sugar, and acid lost is more nearly equal to the total dry weight lost than in Bramley's Seedling. In both varieties some of the hydrolysis products must be soluble, non-reducing substances and accumulate to a small extent (see Widdowson (66) and p. 431).

#### *Loss of Total Dry Material.*

Since sugar forms the greater part of the material lost during storage, the curves representing loss of dry weight will have much the same form after starch has disappeared as those for sugar. The dry weights of the six sets of apples are shown in Fig. 11. In the 1st picking of Worcester Pearmain, where nearly 1 per cent. of starch was present at gathering, the rate of loss of dry weight was very high and nearly constant throughout storage life. In the 2nd picking there was a fall in the rate of loss as starch disappeared, and then it remained constant for the rest of the period observed. No definite evidence was found of an increase in the rate as the reducing sugars began to fall (cf. sugar losses, p. 443).

In the 1st picking of Bramley's Seedling the rate of loss of dry weight also declines as starch hydrolysis nears completion, and then increases again, at first very slowly and then more rapidly as reducing sugars approach the maximum value. In the 2nd picking, where only a little starch was present at gathering, the rate of loss increased slowly from the first observation, and, as in the 1st picking, more rapidly as reducing sugars reached the maximum value. In the 3rd picking, where only traces of starch remained at gathering, this period of very slow rate of loss was much shortened, and in the 4th picking was almost absent.

The mean rates of loss of dry weight for the period subsequent to

starch hydrolysis are shown in Table XVII. The rates of loss while reducing sugars are increasing and decreasing have been calculated for the 2nd, 3rd, and 4th pickings of Bramley's Seedling. In the 1st picking, since

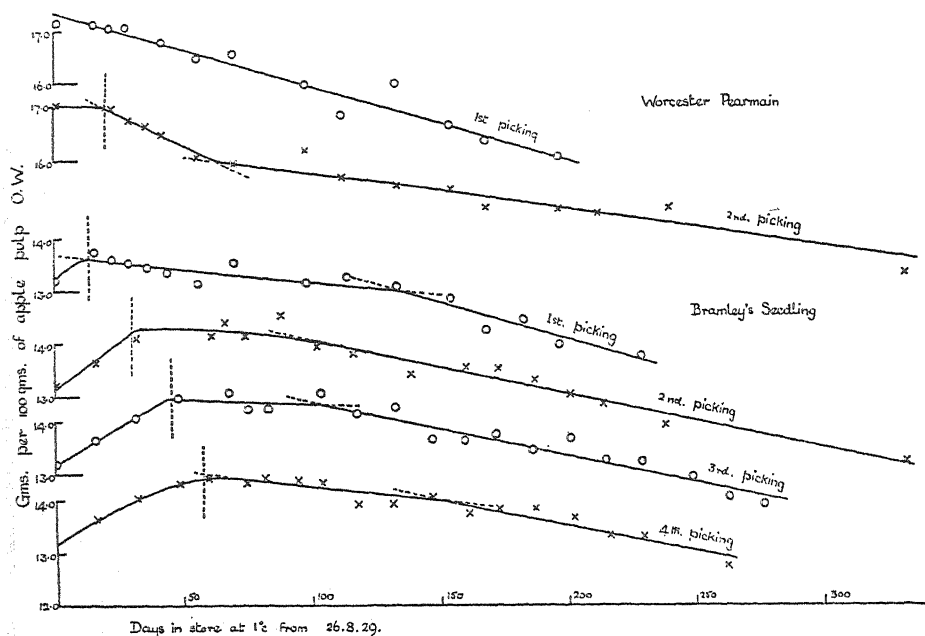


FIG. 11. Quantity-time curves for the dry weight of Bramley's Seedling apples gathered at four different dates, and for Worcester Pearmain apples gathered at two dates, during storage at 1° C. Vertical dotted lines mark the gathering time of each picking, and observations to the left of these lines are the values of dry weight of samples picked in the weeks before gathering. Calculated lines of closest fit from which rates of loss have been determined are continued beyond points of intersection by dotted lines.

reducing sugars are increasing for nearly the whole storage period, values have been found for both the 1st and 2nd 100 days in store. The time of starch hydrolysis is included in the calculations for the 1st and 2nd pickings. For the 2nd picking of Worcester Pearmain the rates of loss were found for the period of starch hydrolysis and subsequent to this time, and for the 1st picking a mean value for the whole storage period; these values are shown in Table XVIII. The calculated lines are drawn in Fig. 11. The lie of the points round the line through the observations for the 1st 100 days in store of the 1st picking of Bramley's Seedling show that there is probably a fall in the rate of loss in the 1st weeks of storage (see p. 451).

The smallest significant difference between the rates of loss in any two pickings was 0.0017 gm. per 100 gm. O.W. per day. Of the values in Table XVII, therefore, that for the 2nd picking is significantly different from those for the 1st and 4th (cf. corresponding results for the sugar lost). The rate of loss in the 1st picking of Worcester Pearmain is significantly



higher than that of the 2nd picking. The rate of loss of dry weight in Bramley's Seedling thus probably falls from the 2nd to the 4th picking and is also low in the 1st.

TABLE XVIII.

*Rate of Loss of Total Dry Weight in Bramley's Seedling and Worcester Pearmain Gathered at Different Dates and Stored at 1° C.*

Rate of loss of dry weight in grm. per 100 grm. O.W. per day.

Worcester Pearmain, 1st picking	(a) 0.01388	
2nd picking	(b) 0.02431	(c) 0.00735
Bramley's Seedling, 1st picking	(d) 0.00517	(e) 0.01369
2nd picking	(f) 0.00452	(g) 0.01042
3rd picking	" 0.00341	" 0.01072
4th picking	" 0.00719	" 0.01021

(a) Mean rate of loss throughout storage.

(b) During starch hydrolysis.

(c) After starch gone.

(d) First 100 days in store.

(e) Second 100 days in store.

(f) While reducing sugars are increasing.

(g) While reducing sugars are decreasing.

The increase in rate of loss in the second half of storage life is significant in all four pickings. Some of the rather marked differences in the rates of loss in the first half of the storage period are due to the inclusion of the period of starch hydrolysis (when present) in these estimates of loss. From these results it is concluded that starch hydrolysis in cold store, which proceeds at a decreasing rate, is accompanied by a falling rate of total loss of dry weight unless but little starch is present at gathering. Sugar consumption begins a little before complete disappearance of starch, and is accompanied by a very slow increase in rate of loss. The general downward trend of loss of dry weight does not begin until a little time after starch disappearance.

The course of loss of dry weight is not affected by gathering the fruit (see Fig. 11), although the average rates of loss are a little different in the four pickings of Bramley's Seedling. The very early-gathered Worcester Pearmain (1st picking), however, did not show a definite fall in the rate of loss as starch disappeared. It has already been pointed out that this set of apples was picked at a physiologically earlier stage than any of the Bramley's Seedling sets.

From this study of the chemical changes in store of the fruit of the two varieties it may be concluded that, if the fruit is left on the tree, maturation changes occur which result in definite differences in the subsequent changes in the sugars in store. In particular, late gathering is associated with a low rate of consumption of sugars in spite of a high rate of inversion of sucrose. Sucrose inversion almost ceases at a higher

concentration in late pickings, and there is also a small increase in glucose content.

Consideration of these data, together with those of the developing apple, suggest that fructose is the end-product of sugar changes in ageing tissue from which no translocation occurs. Both the fact that in Worcester Pearmain fructose appears to be the only product of starch hydrolysis in the last stage of ripening, and that in both varieties in cold store fructose accumulates as a result of sucrose inversion, seem definite evidence of the conversion of glucose to fructose in the apple. It may be tentatively suggested that glucose in the process of conversion (possibly by means of hexose phosphate) to fructose is the most readily oxidizable substrate for respiration, while the rate of conversion to the more stable form of the fructo-furanose produced by inversion is very rapid in comparison with the oxidation rate. When inversion is producing more than twice as much sugar as is required for oxidation, all the fructose is stored, and the excess glucose is converted to fructose. As the inversion rate declines, all the glucose is oxidized in process of conversion to fructose and some fructose as well. On the assumption that the formation of fructo-pyranose is very rapid, it would be this stable form which is oxidized. This form of fructose is certainly oxidized in the later stages when sucrose no longer supplies enough sugar. There is no direct evidence for the consumption of fructo-furanose, and stored glucose is only doubtfully consumed even after prolonged storage (see Fig. 9).

It has been shown that glucose tends to increase in late-gathered Bramley's Seedling, but there is no evidence of any increase in Worcester Pearmain. There may, therefore, be a difference in the rate of conversion of the sugars to one another depending on both age and variety. The increasing rate of sugar oxidation in Bramley's Seedling appears to be associated with an increasing proportion of fructose in the sugar lost, and sugar loss should probably be represented as increasing continuously throughout storage life. It must be remembered that the straight lines shown in the figures are only used to obtain estimates of the mean rates of loss over any given time. The part played by acid in the oxidation mechanism is obscure. The loss of acid seems to bear a definite ratio to the loss of sugar in each variety (see Table XVII).

Although no 'breakdown' appeared in this fruit the changes associated with late gathering, namely a high rate of sucrose inversion coupled with a low rate of sugar oxidation and a high 'minimum' concentration of sucrose, are those associated with bad keeping properties by Haynes and Archbold (see 23) and by Kidd and West (see 23). It must, however, be concluded that these factors do not determine 'breakdown', since none appeared here. Changes in the nature of the carbohydrate oxidized may be a factor in determining the time of the onset of 'breakdown' in susceptible fruit, but

they cannot be regarded as a principal factor in determining susceptibility. Wallace (63) has found that the 'core flush' type of fruit is converted to internal breakdown type by manurial treatment, and recently Pilling and Pearsall (51) have suggested that protein breakdown may be an important factor in determining susceptibility. Both these observations point to the possible importance of nitrogen metabolism. It is of interest that the least susceptible fruit is generally that from trees which have not received manurial treatment, and in which the nitrogen content is low.

#### SUMMARY.

The changes in dry weight, total nitrogen content, sugars, acid, starch, and other alcohol-insoluble material in the pulp of the fruit of two varieties of apple (Bramley's Seedling and Worcester Pearmain) during growth, and the effect of time of gathering on the changes in these constituents during subsequent storage at 1° C. have been investigated.

The samples were chosen so that suitable data should be available for statistical tests of the significance of observed differences in the rates of change of the various constituents.

The method of preparation of solutions for sugar estimations is described in full, and references are given to the detailed description of the other analytical methods employed. For the estimation of sugar in the developing apple a method specially adapted to the determination of small quantities was used.

#### *Changes during Growth.*

Total growth rate (mean increase in weight per apple) was found to increase for the first three weeks of development, and then remained constant until the fruit was gathered. Worcester Pearmain were gathered at a stage much earlier physiologically than Bramley's Seedling, and while still increasing rapidly in size.

In one instance (Bramley's Seedling 1930) fruit was left on the trees as long as possible, and growth ceased abruptly at the end of October, although some fruit still remained on the trees at the end of November.

No starch was present during the first three weeks of growth in either variety, and 53 per cent. of the material, other than water, accumulating in the fruit, was stored as insoluble material and acid during this time. The more acid Bramley's Seedling stored less insoluble material and more acid, while the Worcester Pearmain stored little acid and more insoluble material, suggesting some relation between these two constituents.

In the first three weeks 15 per cent. of the solids were stored as sugar; fructose and sucrose being in nearly equal amounts, and glucose slightly in excess of the other two. 30 per cent. of the solids remained unestimated.

Starch synthesis began after 22 days, and lasted about 60 days in Worcester Pearmain and 30 in Bramley's Seedling. During this time the percentage of solids stored as sugar increased to 55, of which 25 to 33 was fructose; that stored as acid and insoluble material decreased to about 17 per cent., while only about 4 per cent. was not estimated.

During the final stage of ripening, in which starch hydrolysis takes place, over 80 per cent. of the solids were stored as sugar, and only 14 per cent. as acid and insoluble material. The amount of sugar produced by starch hydrolysis was small in comparison with that accumulating in the fruit, and no marked fluctuation in the rate of increase of sugar was found.

The rate of nitrogen intake reached a maximum value after 2 weeks development, and then decreased continuously.

The relationships between the various constituents and the possible nature of the unestimated material are discussed.

#### *Effect of Time of Gathering on the Rate of Chemical Change in Store.*

Bramley's Seedling apples are generally gathered when containing only a little starch, but Worcester Pearmain may contain at gathering up to 1 per cent., and are picked at a physiologically earlier stage than the acid varieties.

Fruit gathered while still containing 0.5 per cent. or more starch shows a decreasing rate of loss of total dry material until nearly all the starch has disappeared, when the rate increases again, slowly at first, and then more rapidly. If only a little starch is present at gathering, the initial rate of loss of dry weight is very low, and increases only slowly at first. During starch hydrolysis sugar content rises (unless only a little starch is present), reaching a maximum value just before starch disappears.

At or near the time of starch disappearance sucrose begins to decline, and total sugar falls slowly. Sucrose inversion proceeds at a declining rate throughout storage life. At first the rate of inversion exceeds that of sugar consumption and reducing sugars rise; during this stage the rates of total dry weight loss and of total sugar loss increase. When sucrose inversion fails to supply sufficient sugar for oxidation, reducing sugars fall, and the rates of total loss and sugar loss increase more rapidly, and then probably fall again after some time.

Fructose is the sugar accumulated when reducing sugars rise, and is also the sugar lost when reducing sugars fall. It is suggested that glucose produced by inversion is oxidized in process of conversion to fructose and, when sufficient glucose is not available, stable fructose is drawn upon. The increasing rate of sugar consumption is associated with an increasing proportion of fructose in the sugar lost. Glucose remains constant or increases slightly during storage.

Acid is lost continuously, and also some alcohol-insoluble material.

Only part of the hydrolysis products of the insoluble material is oxidized, the rest appears to accumulate as a soluble non-reducing substance, which was not determined.

Late gathering is associated with a low average rate of total sugar loss, a high rate of sucrose inversion, and a high level of concentration at which sucrose inversion nearly ceases. The changes in reducing sugar are also greater in late-picked fruit, and the initial acid concentration is low. The rate of acid lost in Bramley's Seedling and in Worcester Pearmain was about the same, in spite of the great difference in concentration.

No internal breakdown occurred in any of the pickings, so that changes in the nature of the carbohydrate oxidized do not appear to be a principal factor in determining susceptibility to this disease, although such changes may determine the time of the onset of breakdown in susceptible fruit.

The author has pleasure in expressing her thanks to Dr. K. Barratt for the facilities afforded her in the collection of material, to Miss E. M. Widdowson and Mr. R. V. Martin for the care with which they carried out much of the analytical work, and to Professor V. H. Blackman and Dr. D. Haynes for their continued interest and advice.

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# Nuclear Division in Plasmodia of *Physarum*.<sup>1</sup>

BY

FRANK L. HOWARD.<sup>2</sup>

With Plate XV and three Figures in the Text.

THE manner of increase in the number of nuclei in large assimilative plasmodia of endosporous Myxomycetes, whether by mitosis, amitosis, or both, is still a somewhat unsettled question. During work on this phase of the life-cycle, the writer has found evidence that division is indeed by mitosis, and the purpose of this paper is to present these results and to describe the process in detail.

References in the literature relating to nuclear division in plasmodia are meagre in detail and somewhat confusing. This state of affairs is due apparently to one or more of the following: (1) the lack hitherto of sufficient material with which to work; (2) an insufficient knowledge of the behaviour of the plasmodium on media; and (3) the dearth of systematic cytological examination of the growing plasmodium. The first difficulty has been overcome since the writer has developed a method of cultivating plasmodia of some endosporous Myxomycetes in large quantities in the laboratory on rolled oat agar, thus providing a prolific, ever-available source of material under conditions favourable for observations and experimentation. Secondly, macroscopic and microscopic observation of the habits of many plasmodia have forced the author to recognize a distinction between growth, mere spread, and forward movement of the plasmodium; three activities, the dissimilarity of which must be taken into account in the search for nuclear division. Thirdly, persistent repeated cytological investigation by the writer has yielded the details of mitosis, and has shown that this process is quickly completed and occurs practically simultaneously, but at no regular interval of time, thus explaining why mitosis has been so difficult to find.

## REVIEW OF LITERATURE.

The relatively scanty evidence presented in the literature on the cytology of the plasmodium furnishes a clue to the contradictory opinions

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regarding the process of nuclear division. After the study and naming of the plasmodium by Cienkowski (1) in 1862, over thirty years elapsed before nuclear division was reported by A. Lister in 1893. Meanwhile, the presence of definite nuclei in a plasmodium had been proved by Schmitz (12) in 1879. His preparations, made by killing the material with osmic acid and staining with haematoxylin, disclosed a large number of nuclei distributed in the plasm, and from observation of these Schmitz concluded that following coalescence of the myxamoebae the previously reported dissolution of the nuclei did not take place.

In 1893 A. Lister (6), having available an abundance of the plasmodium of *Badhamia utricularis*, undertook the task of determining the type of nuclear division. He writes, 'The examination of over a hundred stainings of streaming plasmodium in which we know that the nuclei have multiplied in vast numbers, and in which no indication of karyokinesis occurs, leads to the conclusion that they increase by simple division.' In the light of the facts brought out in the present paper, i.e. the rapidity of the mitotic division and the probability that the nuclei increase in geometrical instead of arithmetical progression by successive divisions, it is little wonder that A. Lister reached this conclusion.

J. J. Lister in 1893, after making preparations from a streaming plasmodium of *B. utricularis* reactivated from a sclerotium, found the nuclei dividing by mitosis, and in a footnote to A. Lister's paper (6) these results are presented as follows: the plasmodium spread itself in large fans over a thin cover-glass, four of these films connected by veins of plasmodium were taken at the same time, killed with Flemming's fluid, and stained. Two of these mountings showed the nuclei in a resting condition while '... in the other two mountings every nucleus is dividing by karyokinesis, some are in the spindle stage, in other parts of the preparation the nuclear plate has divided and the daughter nuclei are in different degrees of separation.'

In 1906 A. Lister (7), after a great number of observations with different species, reiterated his conclusion that during the active life of the plasmodium, when the nuclei multiply enormously, they divide by constriction, or by simple division, pointing out that mitosis had only been found once and raising the questions as to how frequently this occurs, and '... what precedes or follows this exceptional process of division, if it be exceptional at this stage?'

In a treatise on the Mycetozoa in 1909 J. J. Lister (10) states that it is his belief that the number of nuclei in plasmodia increases in two ways: (1) by simultaneous mitotic division, and more commonly (2) by multiplication through simple division. He points out that the multiplication by simple division is difficult to observe owing to the movement of the plas-

modium, but cites, as evidence that amitosis is a frequent occurrence, an experiment in which samples of a plasmodium of *B. utricularis* were fixed and stained every quarter-hour for fourteen hours, during which time the plasmodium increased in size about fourfold. In the fifty-six samples thus obtained, the nuclei were found to be approximately equally abundant in all, and presented considerable difference in size, but in none was there any indication of mitotic division. When drawing his conclusions, J. J. Lister assumed that mitotic division in the growing plasmodium requires one to one and one-half hours as it does prior to spore formation, and that it is simultaneous. Accordingly, he concluded that mitosis had not occurred during the fourteen hours of observation, although the number of nuclei appeared to have increased fourfold in this period, and he explained this multiplication of nuclei by simple division. Certain fallacies in the above assumption as to the amount of increase of the plasmodium, and the time and type of division, will become obvious as the reader considers the observations presented in this paper, and for that reason they are taken up later in the discussion.

Another report of mitosis in the macroscopic plasmodium has been made by Jahn (5), who found nuclear division twice in growing plasmodia of *B. utricularis*; once in 1907, and again in 1910. In the investigation of 1907, he took samples at half-hour intervals from 10.30 a.m. until 6 p.m., and the sample taken about 12.30 p.m. showed nuclear division, even though the division stages were unfavourable for detailed study. In the investigation of 1910 he took samples at fifteen-minute intervals from 9 a.m. until 8 p.m., and found 'dass während dieser Zeit von 11 Stunden die Kerne des Plasmodiums sich einmal, und zwar auch des Mittags gegen 1 Uhr, karyokinetisch geteilt hatten.' As a result of his studies, Dr. Jahn finds no evidence of amitosis, but finds on the contrary that mitosis does occur; results which the writer in the present paper corroborates and supplements with descriptions and illustrations of the details of the process.

G. Lister (9, 8), in discussing the rapidly growing macroscopic plasmodium, reports the nuclei dividing by mitosis simultaneously over a wide extent after an examination of slides prepared by some of the preceding investigators. She says further, '... considering the many prolonged and unsuccessful attempts that have been made to observe this process, it seems not improbable that they also sometimes divide by direct division'.

Finally, Schünemann (13), in studying the sexuality and life-history of *Didymium nigripes*, cut small pieces simultaneously from large plasmodia in five or six Petri plates at hourly intervals, fixed them in Gilson's fluid, stained with iron-alum-haematoxylin, and mounted them in Canada balsam. Later, the samples undergoing mitosis were dissolved out of the balsam, embedded in paraffin, and sectioned  $3\mu$  thick. Upon examining the

preparations Schünemann found the division figures not to be in the same stage, since small pieces of plasmodium contained metaphase, anaphase, and telophase figures close to one another. He observed the nuclear membrane present in the prophase and metaphase figures, and remarks that most of the spindles are arranged in polar view, due possibly to protoplasmic streaming.

From the foregoing review of the discussions upon which is based the present knowledge of the phenomenon of nuclear division in the macroscopic plasmodium of an endosporous myxomycete, it is obvious that mitosis has been found by three observers: J. J. Lister (6), Jahn (5), and by Schünemann (13) in the large plasmodia of two endosporous species, *B. utricularis* and *D. nigripes*. The rarity with which mitosis has been found has tended to support the view that the nuclei may also divide by direct or amitotic division. Opposed to this is the positive statement of Jahn (5) that, 'Es ist merkwürdig, das ich bei der Durchmusterung dieser vielen Plasmodienpräparate niemals etwas von der direkten Kernteilung gesehen habe, die Lister beschrieben hat.' In agreement with Jahn's view the writer has never observed a clear-cut case of amitosis in the thousands of nuclei which have been examined in many hundreds of preparations of *Physarum* plasmodia.

#### MATERIAL AND TECHNIQUE.

Plasmodia of *Physarum polycephalum*, Schw., grown on rolled oat agar, furnished the material for this investigation. The plasmodia were cultivated from year to year, and treated the same as fungus cultures by transferring a portion of the vegetative thallus to fresh plates of rolled oat agar at intervals. On the fresh agar the plasmodial fragment feeds and grows so rapidly that usually the surface of the medium is covered by a streaming plasmodium within two to five days. Both Petri plates and eight-inch moist chambers were used as culture chambers. Plasmodia which were growing as rapidly as possible were chosen for the investigations.

Systematic search for nuclear division was made in both living and killed material. Streaming plasmodia were observed with the microscope for hours at a time, in order that some inkling might be obtained of the time and occurrence of division. The nuclei are so minute that recognition of the process of division in living material is uncertain, therefore, fixed and stained microscopic preparations were depended upon in this study.

Two types of preparations were made; smears and paraffin sections. The smear method was used to expedite the finding of mitosis, while paraffin sections were made for comparative study. Whenever the plasmodium was large enough to permit, duplicate samples were taken from opposite sides at each interval (Tables I and II). The preparations were made from samples one to two centimetres in area, cut at regular intervals

of time from the streaming plasmodium, and immersed immediately in a fixing solution. The fixing fluids used were: Taylor's modification of Flemming's solution (11), Allen's modification (P.F.A.<sub>3</sub>) of Bouin's solution (11), and a chromium trioxide-copper hydroxide solution used by Zirkle (15) in his studies on *Pinus*.

After fixation and washing, smears were made in duplicate on slides from a portion of each sample, and it is worthy of note that in showing details of the mitotic process the smear preparations are equal to the paraffin preparations, so far as the nuclei are concerned. The plasmodial smears were stained with iron-alum-haematoxylin, dehydrated, and mounted in balsam.

In order to have paraffin sections for comparative study larger samples were taken in one case (Plasmodium 9, Table II) in duplicate at each interval, and after using a small portion of each sample for smear preparations to locate mitosis, the remaining portion of the samples which showed the stages of mitosis was embedded, cut into transverse and longitudinal sections  $3\ \mu$  thick, stained with iron-alum-haematoxylin, dehydrated and mounted in balsam.

#### OBSERVATIONS.

The work is for clarity presented separately in two series of trials; in the first series of which the object was merely to ascertain and to describe the manner in which the nuclei divide—mitotic, amitotic, or both (Table I), while in the second series an attempt was made to locate mitosis more definitely by making cytological examinations of portions of the plasmodium where direct macroscopic observation showed growth that would imply nuclear division (Table II).

Having in mind only the finding of mitosis, the majority of the early trials were made during the noonday period (Table I), since Jahn (5) in both instances had found division at this time of day. This seemed a likely time, especially if division occurs periodically at a certain time of the day or night as has been demonstrated for some organisms, or if sunlight in some direct way might influence division in the plasmodium.

After finding from cytological examination of plasmodia subjected to light and darkness that mitosis took place without regard to these conditions, the writer now believes these factors not to influence mitosis. Further, nuclear division has been found not to be periodic, that is, confined to a particular time of day, since the writer has found mitosis occurring at 12.50 p.m., 2.41 p.m., 10 p.m., and 12.15 a.m. (See Tables I and II.)

Fifteen plasmodia were used for obtaining the data in Table I. Bouin's solution was at first used for fixation of the plasmodial samples, but Flemming's solution was used in the later trials as it was found to give a better nuclear picture. The trials lasted from one to thirteen hours, depending

TABLE I.

*Nuclear Division in Plasmodia of Physarum polycephalum*, Schew., 1930.

Number of trial.	Fixative used.	Date of trial.	Time of day.	Length of trial (hours).	Number* of samples.	Interval between samples.	Nuclear condition.
1.	Bouin's	Mar.	11.30 a.m.-3.0 p.m.	3½	8	30 min.	Resting
2.	"	Mar.	11.30 a.m.-3.0 p.m.	3½	8	30 "	"
3.	"	Mar. 24	11.0 a.m.-3.0 p.m.	4	5	60 "	"
4.	"	Apr. 5	11.0 a.m.-12.0 p.m.	13	27	30 "	"
5.	Bouin's and Flemming's	Sept. 1	8.30 p.m.-4.0 a.m.	7½	16	30 "	"
6.	Flemming's	Sept. 19	9.50 a.m.-12.50 p.m.	3	13	15 "	Mitosis at 12.50 p.m.
7.	"	Oct. 3	10.30 a.m.-1.45 p.m.	3¼	14	15 "	Resting
8.	"	Oct. 8	10.27 a.m.-2.10 p.m.	3¾	16	15 "	"
9.	"	Oct. 16	10.0 a.m.-12.15 p.m.	2¼	10	15 "	"
10.	"	"	11.30 a.m.-1.30 p.m.	2	9	15 "	"
11.	"	"	10.0 a.m.-1.45 p.m.	3¾	16	15 "	"
12.	"	Nov. 1	10.0 a.m.-2.0 p.m.	4	**17	15 "	"
13.	"	Nov. 10	1.50 p.m.-4.0 p.m.	2½	14	10 "	"
14.	"	"	4.10 p.m.-3.30 a.m.	11½	**69	10 "	Mitosis 10.0 to 10.30 p.m.
15.	"	Nov. 11	1.41 p.m.-2.41 p.m.	1	13	5 "	Mitosis at 2.41 p.m.

\* Duplicate smears were made from each sample.

\*\* Duplicate samples were taken from opposite sides of the plasmodium.

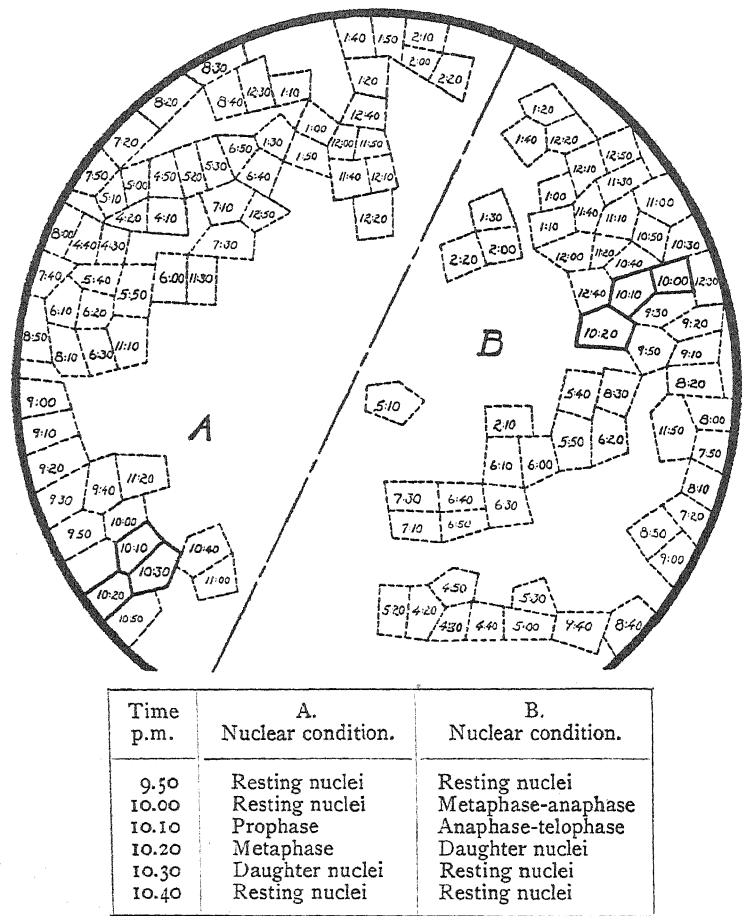
upon the size of the plasmodium and the interval between samples. This table also shows the length of each trial, the time of day, the interval between samples, and the nuclear condition. It will be noted that the interval between samples was reduced from thirty or sixty minutes to five or ten minutes, as it was felt, and subsequently proved, that mitosis might easily be overlooked if samples were taken at intervals of thirty minutes or more.

Unfortunately, records were not kept of the length of time between transfer and the taking of samples, except in the case of Trial 14, where the plasmodium is known to have been growing four days on the plate of agar. Only resting nuclei were found in twelve of the fifteen trials. Twice mitosis was found in the last sample taken (Table I, Trials 6 and 15), and once a complete series showing all stages of the mitotic process was obtained (Table I, Trial 14).

Apparently mitosis is not localized in any one part of the streaming plasmodium, but occurs practically simultaneously throughout. This was found to be the case in Trial 14 (Table I), in which duplicate samples were taken from opposite sides of the plasmodium, which practically covered the surface of the agar. Text-fig. 1 is a chart of this culture in an eight-inch moist chamber, showing the location of the samples taken at ten-minute intervals. To understand the irregularity in the shape and size of the samples in this chart, one must visualize a constantly streaming plasmodium moving over the agar surface of the large moist chamber the diagram represents. The samples in which mitosis was found are outlined in solid black lines, while the table at the bottom of the chart gives the time at which the various stages of division were found. From this it is apparent that nuclear division is not quite simultaneous, but that a ten-minute difference may occur between the two sides of a large plasmodium. The nuclei of a given area, however, were found to be in about the same stage of mitosis, for, although in smear preparations we may find the nuclei in the prophase and metaphase or anaphase and telophase of division, never were all of the phases from prophase through telophase observed in a single field. In paraffin sections, however, where there was no opportunity for the nuclei to become mixed by smearing, all of the nuclei of a particular section are found to be in almost exactly similar stages of mitosis. The table at the bottom of Text-fig. 1 also clearly shows that nuclear division may be completed within a remarkably short time—thirty minutes or less. The rapidity with which the nuclei divide probably accounts in part for the fact that division has been observed so infrequently.

The results of the second series of trials, in which an attempt was made to correlate growth and nuclear division, are summarized in Table II. Thirty-nine plasmodia varying in area from eleven square centimetres to over three hundred square centimetres were used. In all cases the samples were taken at regular ten-minute intervals, and if the plasmodia were large

enough (Table II, Trials 1, 2, 7, 8, 9) duplicate samples were taken from opposite sides at each interval. The length of time the plasmodium remained on the fresh plate of agar before samples were taken may be



TEXT-FIG. 1. Chart showing the time and location of each sample taken in Trial 14 (Table 1), and table giving the time mitosis was found. Reduced one-half.

found in column 4 of Table II. For the tenth trial and part of the ninth, Zirkle's chromium trioxide-copper hydroxide fixing solution (15) was tried, but material thus prepared was found to be more distorted than material fixed with modified Flemming's solution (11).

In an effort to obtain data on which one might predict the time after transfer at which mitosis would take place in a plasmodium, records were kept of the time from transfer to a fresh substratum until the samples were taken. In order to ascertain if such a correlation



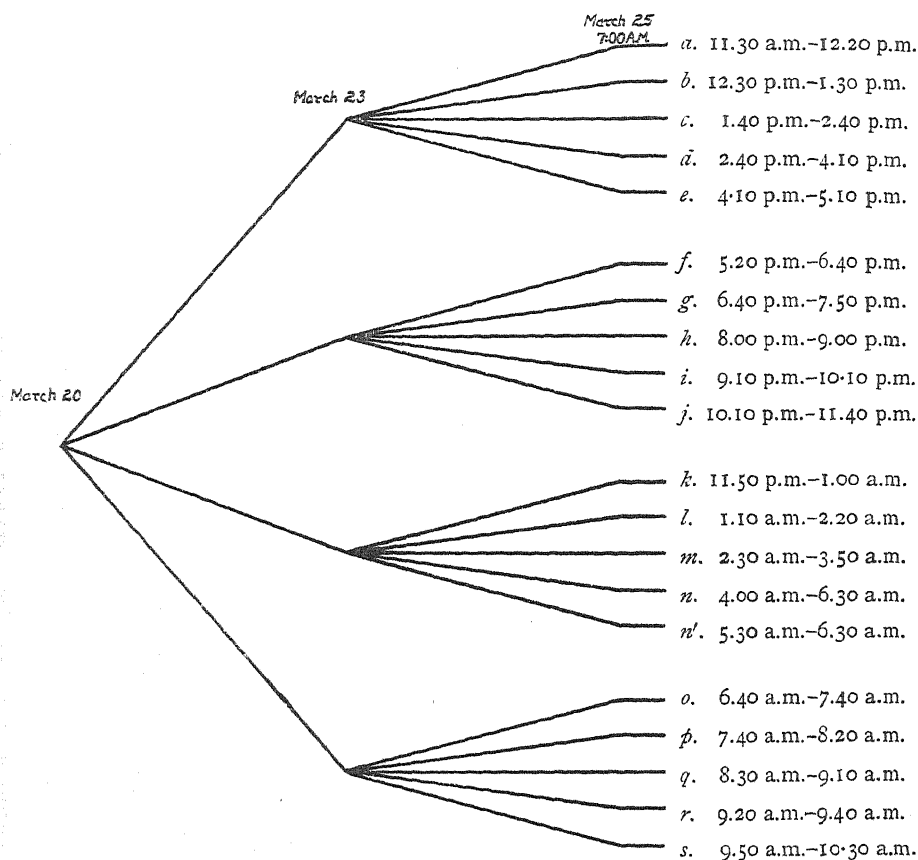
TABLE II.  
*Nuclear Division in Plasmodia of Physarum polycepalum*, Schw., 1931.

Number of trial.	Fixative used.	Date of trial.	Hours elapsing between transfer and taking of samples.	Time of day.	Extent of culture.	Length of trial (hours).	Number* of samples.	Interval between samples.	Nuclear condition.
1.	Flemming's	Mar. 3	72	2.45 p.m.—5.35 p.m.	10 cm. dish	2 $\frac{1}{2}$	**18	10 min.	Resting
2.	"	"	72	2.45 p.m.—5.35 p.m.	"	2 $\frac{1}{2}$	**18	"	"
3.	"	Mar. 25— Mar. 26	4 $\frac{1}{2}$ —27 $\frac{1}{2}$	11.30 a.m.—10.30 a.m.	Twenty 10 cm. dishes	23	139	"	"
4.	"	Apr. 2	21—29 $\frac{1}{2}$	10.0 a.m.—6.30 p.m.	Four 10 cm. dishes	8 $\frac{1}{2}$	52	"	"
5.	"	Apr. 7	27 $\frac{1}{2}$ —36 $\frac{1}{2}$	11.35 a.m.—8.35 p.m.	Three 10 cm. dishes	9	55	"	"
6.	"	"	96—102	5.45 p.m.—11.55 p.m.	10 cm. dish	6 $\frac{1}{2}$	38	"	"
7.	"	Apr. 17	38—46	10.0 a.m.—6.40 p.m.	20 "	8 $\frac{3}{4}$	**53	"	"
8.	"	"	38—51 $\frac{1}{2}$	10.0 a.m.—11.30 p.m.	"	13 $\frac{1}{2}$	**82	"	"
9.	"	Apr. 28	143 $\frac{1}{2}$ —158 $\frac{1}{2}$	9.45 a.m.—9.55 p.m.	"	15 $\frac{1}{2}$	**92	"	Mitosis 12.15 a.m.— 12.45 a.m.
10.	Zirkle's	Apr. 28	13 $\frac{1}{2}$ —15 $\frac{1}{2}$	10.05 p.m.—12.55 a.m. 11.15 p.m.—1.15 a.m.	10 cm. dish	2	13	"	Resting

\* Duplicate smears were made from each sample.

\*\* Duplicate samples were taken from opposite sides of the plasmodium.

exists, the following experiment was performed (Text-fig. 2); four pieces of a rapidly growing plasmodium were each transferred on March 20 to four fresh Petri plates of rolled oat agar. Three days later, on March 23,



TEXT-FIG. 2. Diagram illustrating the derivation of the subcultures from the parent plasmodium in Trial 3 (Table II). On March 20 the parent plasmodium growing on rolled oat agar was divided into four cultures, and on March 23 these were further divided into twenty subcultures. At 7.0 a.m., March 25, the twenty plasmodia were retransferred to fresh plates of oat agar and from these the samples were taken at ten-minute intervals beginning at 11.30 a.m., March 25, and ending at 10.30 a.m., March 26.

five pieces of plasmodium of approximately equal size were cut from each of the four dishes, and the resulting twenty plasmodial fragments were each transferred to a fresh plate of rolled oat agar. These were allowed to grow until 7 a.m., March 25, when a sample from each plate was transferred to a fresh dish of oat agar, and four and a half hours after transferring, or at 11.30 a.m., the experiment was begun. Samples were taken at intervals of ten minutes, starting with culture *a*, and ending with culture *s*. Some overlapping occurred when samples were taken from culture *n* and

culture  $n^1$  at the same time. Only resting nuclei were observed in the preparations made during the twenty-three hours of this experiment. Such results as these, when plasmodia derived from the same parent culture and grown on the same medium under similar environmental conditions are used, seem to indicate that nuclear division does not take place until the plasmodium has assimilated nutrients from the fresh medium.

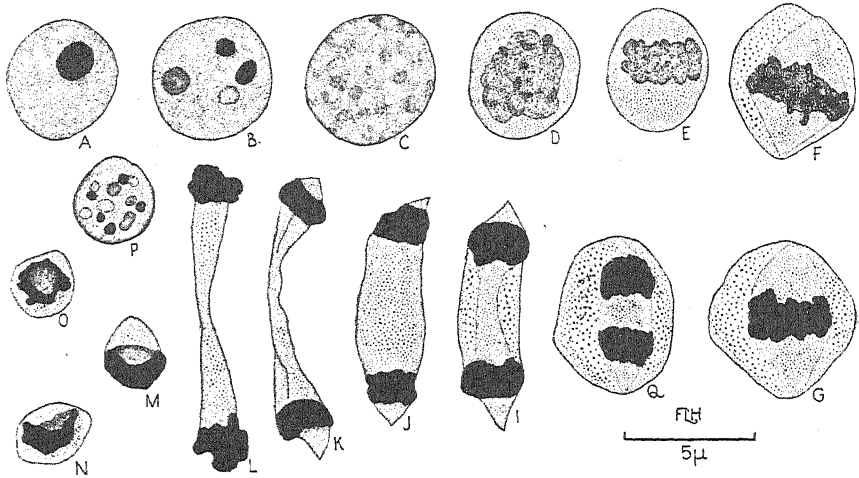
Four and a half hours were allowed to elapse before taking the first sample in order to allow the plasmodium to move at least partially off the piece of agar upon which it has been transferred, and thus to be in a position to absorb nutrients from the fresh rolled oat agar, since repeated observations have shown that the plasmodium generally moves from the transfer piece of agar within two to six hours, and continues to move and to spread over the fresh agar surface for about the first twenty-four hours. Generally after about twenty-four hours the plasmodium lessens its movement, and begins to absorb the oat kernels embedded in the agar. Thus it is the second or third day after transferring that the plasmodium is actually absorbing nutrients and growing. Subsequent experiments were carried out (Table II, Trials 4, 5, 7, 8) in which the samples were taken at ten-minute intervals up to fifty-one hours after transfer, but in preparations made during this time nuclear division was never observed. The chance of finding mitosis, therefore, seems more likely after the plasmodium has assimilated nutrients. In substantiation of this, reference to Table I (Trial 14) and Table II (Trial 9) will show that mitosis was not found in the plasmodium until the fourth and seventh day respectively after transfer. Although samples were taken in Trial 10 (Table II) during the same period that mitosis was taking place in Trial 9 (Table II), there was no evidence of mitosis.

In addition to examination of killed and stained plasmodial preparations, the behaviour of the streaming plasmodium was followed macroscopically by estimation of any increase in size, and by direct microscopic observation. It was hoped that cessation of streaming might be connected with mitosis, but macro- and microscopically the streaming plasmodium presents no feature, as yet discernible, indicative of nuclear division. Although observation of the growth of a plasmodium assures the investigator that an increase in volume has taken place, and consequently it follows that nuclear division must occur, yet the precise ratio of cytoplasm to nucleoplasm at which division occurs is still unknown.

#### DETAILS OF MITOSIS.

Somatic mitosis in the macroscopic plasmodium resembles in many respects the type found in vascular plants, since the achromatic figure is devoid of asters and centrosomes. The process differs in some details, however, as the accompanying illustrations in Text-fig. 3 and Pl. XV show.

The figures were drawn with the aid of a camera lucida, using a Zeiss 2 mm. apochromatic objective, and a compensating ocular (K30 $\times$ ). Although adjoining paired nuclei seemingly undergoing amitotic division may occasionally be found in stained smears from streaming plasmodia, careful focusing



TEXT-FIG. 3. Camera lucida drawings of the various stages of mitosis as found in the macroscopic plasmodium of *P. polycephalum* made from material fixed with Flemming's solution and stained with iron-alum-haematoxylin (cf. Plate XV). A-B. Nuclei during interkinesis with one or several nucleoli. C-E. Successive stages of prophase showing the concentration of the reticulum. F. Typical metaphase figure surrounded by a distinct nuclear membrane. G and Q. Early anaphase figures showing separation of clumped chromosomes. I. A late anaphase figure showing the usual shrinking away of the nucleoplasm from the membrane to form a cylinder (cf. Plate XV, Figs. 14-16 which show the stainable material commonly present at the equator of the cylinder at this stage). J. Similar anaphase figure but with the less frequent lack of shrinkage. K-L. Telophase just before the nucleoplasmic cylinder breaks. M-P. The clumped chromatin reorganizing to form the daughter nucleus.

of the microscope has invariably shown each nucleus to be surrounded by a distinct membrane. The nuclear membrane is a definite structure which can be clearly discerned through late anaphase (Pl. XV, Figs. 14-16), and is seemingly present throughout nuclear division, making the process entirely intranuclear.

Representative resting nuclei, either with a single large nucleolus or several smaller ones, and with faintly reticulate nucleoplasm, are shown in Text-fig. 3, Figs. A-B, and Pl. XV, Figs. 1-4. Nuclei having two to five nucleoli are common, and may be found at any time during interkinesis, contradicting the idea that nucleolar fragmentation is associated with the initiation of mitotic division. The four photomicrographs of resting nuclei (Pl. XV) also bring out the variation—from 3  $\mu$  to 5  $\mu$ —in the size of nuclei during interkinesis.

The first sign of nuclear division is evidenced by an almost imperceptible granular appearance of the lightly stained nucleoplasm, while the nucleolus is distinct and stains deeply. Within the next ten minutes the

nucleoplasm becomes coarsely granular and stains more intensely, while the nucleolar material becomes indefinite in outline. The nucleolus is seemingly connected with the reticulum by threads, and the nucleolar material apparently is gradually incorporated in the reticulum, since the nucleolus loses its identity as the reticulum becomes more distinct (Text-fig. 3, Fig. C; Pl. XV, Figs. 5-6). The chromatin at late prophase appears in a single plane of focus as coarse granules scattered throughout the nucleus (Text-fig. 3, Fig. C), but by changing the focus it can be discerned as an irregular thread-like condensation in the matrix of less densely staining nucleoplasm. Concurrently, the nucleus is usually found to attain its greatest diameter during the granular prophase stage (Pl. XV, Fig. 6), ranging from  $5\mu$  to  $6\mu$ ; after which the nucleoplasm and chromatin generally shrink, although the nuclear membrane (Pl. XV, Fig. 7) may remain distended for some time.

The deeply staining thread-like material of the spireme aggregates itself at the centre of the nucleus (Text-fig. 3, Figs. D-E; Pl. XV, Figs. 7-8), and soon there ensues a typical metaphase figure (Text-fig. 3, Fig. F; Pl. XV, Figs. 9-12). At metaphase the chromosomes form a compact plate with an irregular outline due to the projecting ends of the chromosomes. The nucleoplasm assumes a spindle-shape, and generally shrinks away from the nuclear membrane which retains its spherical or slightly ellipsoidal outline during metaphase, and even until the clumped chromosomes separate during anaphase, after which the membrane becomes more and more attenuated (Text-fig. 3, Figs. G-L; Pl. XV, Figs. 13-16).

During anaphase the chromosomes tend to separate as two clumps in spite of the use of different fixing fluids and the addition of adjuvants. This, coupled with the small size of the nucleus, makes it extremely difficult to obtain a positive count of the chromosomes. At late anaphase some nuclei have the nucleoplasm shrunk away from the nuclear membrane, while others do not (cf. Text-fig. 3, Fig. I with Fig. J; also Pl. XV, Fig. 13 with Figs. 14-16). Connecting fibres between the separating clumps of chromosomes are absent. In the connecting cylinder of nucleoplasm there appears in some nuclei at this stage, though not in others, a portion of stainable material which may be line-like or may be irregular in equatorial view and of a composition and function unknown to the writer (Pl. XV, Figs. 14-16).

At telophase dark lines sometimes appear to be present in the connecting strand, but, by carefully focusing these, become resolved into wrinkles or folds in the surface (Text-fig. 3, Fig. K; Pl. XV, Fig. 18). Just how the connecting cylinder breaks, allowing the daughter chromosome groups to become separated, could not be made out, perhaps because this may occur almost instantaneously. Many figures with the connecting cylinder of spindle-substance stretched to the breaking-point were found

(Text-fig. 3, Fig. L; Pl. XV, Fig. 18) as were many daughter nuclei shortly after the stretched nucleoplasm had separated (Text-fig. 3, Figs. M-O; Pl. XV, Fig. 19). No relationship could be established between the densely staining material mentioned above (Pl. XV, Figs. 14-16) and the scission of the connecting cylinder of nucleoplasm.

Following separation of the connecting strand the chromatin orients itself in the centre of the daughter nucleus and then begins to fragment, meanwhile the nucleoplasm is rounding up and enlarging (Text-fig. 3, Fig. P; Pl. XV, fig. 20). The fragments of chromatin become more and more disorganized, until the stained nucleus has an unevenly granular appearance without a nucleolus, and it is some time before the nucleolus is again reorganized.

#### DISCUSSION.

Nuclear division in the vegetative thallus of the endosporous Myxomycetes has been previously reported as taking place by amitosis, and by mitosis, while the details of the processes have been somewhat involved in speculation. The observations presented in this paper have given evidence that mitosis only, and not amitosis, is the normal means of the multiplication of nuclei, and have contributed details of the mitotic process.

In studies upon nuclear division in plasmodia the importance of an understanding of the habits of plasmodia on nutrient substrata should be given more emphasis. Since no directly observable criterion indicative that nuclear division is taking place in the assimilating plasmodium is as yet known, and since nuclear division is apparently accomplished at irregular intervals, only by knowing when the plasmodium is growing can success be expected. The natural assumption that growth and nuclear division are related seems to be borne out, and the writer believes that the considerable odds against finding mitosis will be greatly lessened if the investigator will make sure that the plasmodium under experiment is actually growing.

A study of cultures upon nutrient media has shown that after a fragment of plasmodium is transferred to a plate of fresh oat agar, the sequence of events is as follows: the plasmodium *moves from* the agar upon which it was transferred, and *spreads out* into a plasmodial fan on the moist agar surface; the fan *creeps* over the plate of agar for a while, finds a favourable location, and begins to *feed*. It is not until this feeding period that an increase in volume is noticeable. A heaped-up bit of plasmodium, when transferred to a Petri plate of fresh agar, may spread until the plate is covered with a thin film of plasmodium, thus giving the impression of simulating growth, even though none has taken place. Likewise, when plasmodia are allowed to stream on a moist glass plate, or on

non-nutrient agar, or even on some nutrient agars, we find that they move about in search of assimilable food, but are not growing.

Mitosis has been shown to take place throughout the plasmodium almost simultaneously, and to result in a doubling of the number of nuclei. Therefore, one division would give a twofold, or two divisions a fourfold increase in the number of nuclei and a corresponding potential increase in the volume of the plasmodium, and still retain the same ratio of nuclei to cytoplasm as was present in the original piece of plasmodium. The number of nuclei in the assimilative plasmodium increases by geometrical progression at each successive division.

Mitosis has been demonstrated by the writer to be completed within a remarkably short time. Twenty to forty minutes are sufficient for the entire process in an actively growing plasmodium in contrast with thirty to sixty minutes which the writer (2) found to be required for mitotic division preceding spore-formation in the sporangium or to the one to one and a half hours assumed by J. J. Lister (10). Since only very late prophase, metaphase, anaphase, and telophase figures are especially characteristic, the observer might easily pass over the early prophase and reorganizing daughter nuclei believing them to be merely abnormalities. Moreover, because of the rapidity of the process, too great an interval between samples may easily account for the failure to find the characteristic nuclear figures.

Apparently mitosis will occur in spite of constant injury to the plasmodium due to cutting the samples. In two plasmodia mitosis was found in the last sample taken (Table I, Trials 6 and 15). The plasmodia were gradually used up as samples were cut out, until only a single fragment remained in each case, yet when these were fixed, smeared, stained, and examined, mitosis was observed to be taking place.

Mitotic division in plasmodia of *Physarum*, as shown in this paper, is rather unusual in that the nuclear membrane persists throughout as in *Cladophora*, yet the nucleolus disappears and an achromatic spindle is formed. The intranuclear division figure has furnished, according to the report of J. J. Lister (10), another bond in the relationship between the Myxomycetes and the Protozoa, but it must be pointed out as the result of this study that the achromatic figure is of the anastral type; the asters and centrosomes so common in the Protozoa are absent.

#### SUMMARY.

Mitotic nuclear division is reported in the macroscopic plasmodia of *Physarum polycephalum* which were growing on rolled oat agar in the laboratory. A study of the habit of plasmodia on culture media has shown a distinction between growth, spread, and forward movement; three activities, a better knowledge of which will assist investigators in finding

mitosis. Nuclear division by mitosis was found in only four of the fifty-four plasmodia studied cytologically, but in the many hundreds of preparations made during the one hundred and sixty hours of observation, no positive evidence of amitosis could be seen. Mitosis has been found to occur almost simultaneously throughout the plasmodium, to result in a doubling of the number of nuclei, to require only twenty to forty minutes for completion, and to have no definite periodic recurrence. The achromatic figure lacks asters and centrosomes, and division is entirely intranuclear.

The writer is indebted to Professor W. H. Weston, Jun., for the willing suggestions and the helpful criticism made during the progress of this investigation, to the Board of National Research Fellowships in the Biological Sciences for a grant which made this work possible, and to his wife, Dorothy L. Howard, for assistance in the laborious task of making preparations.

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## EXPLANATION OF PLATE XV.

Illustrating Dr. Howard's paper on Nuclear Division in *Plasmodia* of *Physarum*.

The photomicrographs were all made from haematoxylin-stained material using a Zeiss 2 mm. oil-immersion objective and a 30 × ocular, and are magnified approximately 3,500 diameters.

Figs. 1-4. Resting nuclei, illustrating variation in their size and in the number of nucleoli they contain.

Figs. 5-7. Nuclei in prophase, showing the characteristically greater diameter of the nucleus and the distinct nuclear membrane which gives the nucleus a cell-like appearance.

Figs. 8-9. Formation of the spindle in the nucleus during early metaphase.

Fig. 10. Polar and spindle views of typical metaphase figures.

Fig. 11. An unusual metaphase figure occupying only a portion of the nucleus.

Fig. 12. Adjacent metaphase and telophase figures from a smear preparation.

Fig. 13. Anaphase figure showing the mantle distinctly, the dark clumped chromosomes, and the nucleoplasm filling the connecting cylinder.

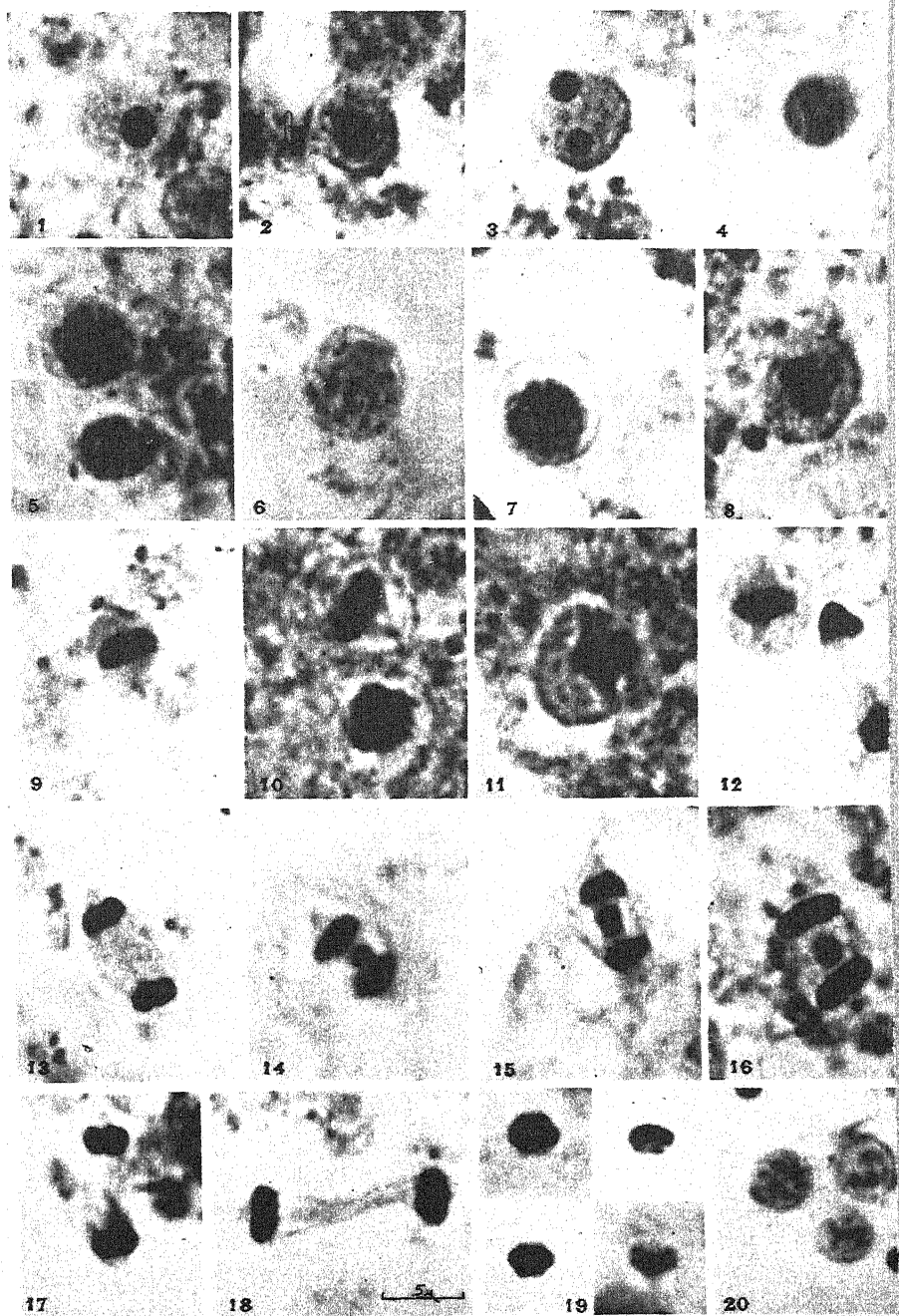
Figs. 14-16. Late anaphase figures demonstrating the darkly staining material commonly present across the shrunken nucleoplasmic cylinder at this stage. Observe that the nuclear membrane is still visible.

Figs. 17-18. Telophase figures, the lines shown in the connecting strand of nucleoplasm prove on focusing to be merely folds in the surface and not to be connecting fibres.

Fig. 19. Later stages showing clumps of chromosomes from four dividing nuclei after the connecting strand has broken and before the daughter nucleus has rounded up (cf. Text-fig. 3).

Fig. 20. Reorganized daughter nuclei showing the more finely divided chromatin material into which the clumped chromosomes have separated, (cf. Text-fig. 3, Fig. v).







# Cytological Studies of some Wheat and Aegilops Hybrids.

BY

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With seventy-seven Figures in the Text.

THE present communication deals with the cytology of a number of hybrids between (1) some varieties of wheats, (2) species of *Aegilops* and wheats, and (3) between species of *Aegilops*.

The following is a list of the hybrids concerned :

			Chromosome numbers of the parents.	
			n.	n.
(1)	<i>Triticum monococcum</i> , L. ♀ var. <i>flavescens</i>	× <i>Triticum aegilopoides</i> , Bal. ♂ var. <i>Thaoudar</i> .	7 × 7	
(2)	<i>T. dicoccum</i> , Schüb. ♀ ♂ var. <i>Timopheevi</i>	× <i>T. aegilopoides</i> , Bal. ♂ var. <i>Thaoudar</i>	14 × 7	
(3)	<i>Aegilops ovata</i> , L. ♀	× <i>Aegilops triaristata</i> , Willd. ♂	14 × 14	
(4)	<i>A. uniaristata</i> , Vis. ♀	× <i>A. Heldreichii</i> , Holzm. ♂	7 × 7	
(5)	<i>A. uniaristata</i> , Vis. ♀	× <i>A. umbellulata</i> , Zhuk. ♂	7 × 7	
(6)	<i>A. caudata</i> , L. ♀	× <i>A. triaristata</i> , Willd. ♂	7 × 14	
(7)	<i>A. caudata</i> , L. ♀	× <i>Triticum durum</i> , Desf. ♂ var. <i>hordeiforme</i>	7 × 14	
(8)	<i>A. caudata</i> , L. ♀	× <i>T. vulgare</i> , Host. ♂ var. <i>lutescens</i>	7 × 21	
(9)	<i>A. triaristata</i> , Willd. ♀ (n = 14)	× <i>T. vulgare</i> , Host. ♂ var. <i>milturum</i>	14 × 21	
(10)	<i>A. triaristata</i> , Willd. ♀ (n = 21)	× <i>T. vulgare</i> , Host. ♂ var. <i>milturum</i>	21 × 21	
(11)	<i>A. triaristata</i> , Willd. ♀ (n = 21)	× <i>T. durum</i> , Desf. ♂ var. <i>leucurum</i>	21 × 14	

From the results of previous investigations,<sup>1</sup> it was concluded that the type of mating as shown in the bivalents at metaphase of the hetero-type division or earlier stage of meiosis when the individual chromosomes are quite clearly defined, is correlated with the difference of relationship between the parents as recognized by the taxonomist.

<sup>1</sup> Percival, John : Cytological studies of some hybrids of *Aegilops*, sp. × wheats, and of some hybrids between different species of *Aegilops*. Journ. Gen., xxii. 201, 1930.

The parents of the several crosses in the list given above exhibit different degrees of taxonomic relationship, and the cytological investigations now recorded were directed chiefly to the determination of the number and type of bivalents found in these hybrids. Wherever the material was suitable, an attempt was also made to trace the nuclear changes leading up to the formation of the univalents and bivalents seen at the metaphase of the heterotype division of the pollen mother-cells.

The technique, method of fixation, and staining were the same as described in a former communication.<sup>1</sup>

#### TERMINOLOGY.

A note is necessary here regarding the connotation of some of the terms used in this paper.

*Synizesis* (συνίσις, synizesis, a collapse). This term is here used in its literal sense for the knotted, contracted state of the nuclear contents at any stage of the heterotype division, and not alone for the contraction commonly observed in early prophase. It does not appear possible to recognize with certainty a first, second, third, or other order of contraction, as is sometimes done, nor does a study of the successive stages of meiosis frequently seen in different pollen mother-cells within the same anther loculus make it certain that such contractions are to be attributed to the action of the fixatives used.

*Syndesis* (σύνδεσις, syndesis, a binding together). The pairing of whole chromosomes (univalents), most clearly recognized at metaphase of the heterotype division or at diakinesis, *acrotyndesis* (ἄκρον, *akron*, tip or end) denoting an end-to-end union of the two components of a bivalent, the pair being arranged in an approximately straight line at metaphase. Although in these hybrids the evidence appears to point to the conclusion that all bivalents are formed by the end-to-end pairing of whole chromosomes, the term *parasyndetic* is retained for those bivalents whose two components are either (1) arranged side by side in close contact along their whole length, (2) joined at both ends and appearing as an open link, or (3) united at one end but bent round so that the bivalent has the appearance of a more or less open horseshoe. (Since the Greek τέλος, *telos*, denotes end in the sense of end of an action, or goal, rather than tip or end of an object, the term *telosyndesis* for *acrotyndesis* or end-to-end pairing is avoided.)

*Univalent*. A single chromosome as observed at metaphase of the heterotype division in these hybrids, the half of a longitudinally divided univalent being termed a *monad*; a single chromosome is a *dyad*, a bivalent a *tetrad* structure.

<sup>1</sup> See footnote, p. 479.

1. *Triticum monococcum* var. *flavescens* ♀ × *T. aegiloides* var. *Thaoudar* ♂.  
( $n = 7$ ) ( $n = 7$ )

The cultivated *T. monococcum* so closely resembles the wild *T. aegiloides* in morphological characters that there need be no hesitation in regarding the former as being derived from the latter.

The gametic chromosome number of both parents is seven and, at the metaphase of the heterotype division of the hybrid, the number of bivalents is also seven, all the parental chromosomes finding mates.

In the majority of cases all the bivalents are of the parasynthetic ring or slightly open ring type as seen in Figs. 6-8; in late metaphase some of the bivalents appear to have opened out as in Fig. 8. Just preceding the metaphase the bivalents are observed to unfold from a dense synizetic knot when the spindle begins to form (Figs. 4, 5). In an earlier stage (Fig. 3) are seen a small number (7?) of pachytene loops or strands, the antecedents of the seven metaphase bivalents; each strand is itself clearly double and traceable back to an irregular and discontinuous spireme of thin double zygotene threads (Figs. 1, 2).

The anaphase of the heterotype division follows the normal course, 7 univalents passing undivided to the poles of the spindle with no sign of lagging chromosomes (Figs. 9, 10); interkinesis is also normal, and the hybrid is fertile, 50 per cent. of the spikelets producing ripe grain.

2. *T. dicoccum* var. *Timopheevi* ♀ × *T. aegiloides* var. *Thaoudar* ♂.  
( $n = 14$ ) ( $n = 7$ )

The female parent is a wheat found in Georgia, Transcaucasia, by Zhukovsky, who considered it of separate specific rank, naming it *T. Timopheevi*: from a study of its morphology and a comparison with other wheats, I classify it as a well-marked variety of *T. dicoccum*.

In vegetative characters both parents closely resemble each other in having markedly hairy nodes and leaves clothed with long coarse hairs unlike those on any other form of wheat.

The  $F_1$  plants are intermediate in inflorescence characters and completely sterile.

The gametic chromosome numbers of the parents are fourteen and seven respectively, and at metaphase of the heterotype division seven bivalents and seven univalents are present, the former arranged on the equatorial plate, the univalents being frequently off the spindle and scattered irregularly in the cytoplasm. In most cells both parasynthetic and acrosynthetic bivalents are seen, from 5-7 of them belonging to the first-mentioned type (Figs. 11, 12),

The components of the bivalents separate normally to the poles of the spindle, but these, as well as the unpaired and scattered univalents, show

the homotypic split in the anaphase of the first meiotic division (Fig. 13); a variable number are always found lagging in the equatorial zone after the rest of the chromosomes have moved to the poles (Fig. 14).

From the polar view of the homotypic metaphase (Fig. 15) it would appear that 10 chromosomes have moved to one pole and 11 to the other.

The final division of the mother-cells is irregular, and pollen-grains of different sizes and chromosome-content are produced.

3. *Aegilops ovata* ♀ × *A. triaristata* ♂.  
( $n = 14$ )                      ( $n = 14$ )

These two species belong to the same section of the genus *Aegilops*, and so closely resemble each other in many morphological characters that some taxonomists look upon *A. triaristata* as a variety of *A. ovata*. Both have the same gametic chromosome number.

The hybrid proved completely sterile.

At the metaphase of the heterotype division, unpaired univalents are present with 6-7 bivalents, the latter of both types, 2-4 being 'parasynthetic', the rest acrosynthetic (Figs. 16, 17).

The movement and distribution of the chromosomes during meiosis is the same as that of *Aegilops* and *Triticum* hybrids generally, in which both paired and unpaired univalents are present at metaphase, lagging chromosomes being found at anaphase both of the heterotype and homotype divisions (Fig. 18).

For comparison the metaphase of the heterotype division of *A. ovata* is given in Fig. 19. Here, as expected, all the chromosomes are paired; in some cells the bivalents are all of the 'parasynthetic' type, while in others a few are acrosynthetic.

4. *A. uniaristata* ♀ × *A. Heldreichii* ♂.  
( $n = 7$ )                      ( $n = 7$ )

Both species of this cross are included in the same section of the genus *Aegilops*: both have the same chromosome number.

At the heterotype metaphase from 5-7 bivalents are found with 0-4 unpaired univalents; an occasional Y-shaped trivalent is also observed. The bivalents are of both types, usually two or three 'parasynthetic', the rest acrosynthetic (Figs. 20-2). Rarely are more than one or two chromosomes found lagging at anaphase (Fig. 24).

5. *A. uniaristata* ♀ × *A. umbellulata* ♂.  
( $n = 7$ )                      ( $n = 7$ )

Like the preceding hybrid the gametic numbers of the parents are the same, namely seven; the parents, however, belong to different sections of the genus *Aegilops*.



In some cells 14 unpaired univalents are seen, while in others from 1-4 bivalents are present, all of the acrosyndetic type (Figs. 31-4).

A more complete investigation was made of the heterotype division in this hybrid, attention being given to stages both earlier and later than the metaphase.

In the stage preceding the clearly defined metaphase, the chromosomes are seen collected into a close knot imbedded in dense cytoplasm with no sign of a nucleolus (Fig. 30).

In the earliest prophase observed the nuclear cavity contains fine discontinuous leptotene threads, varying in length; their number cannot be counted with certainty, but all are single and no sign of a split can be discerned, even under the highest magnification (Fig. 25). In the stage immediately succeeding this, the threads are thicker and double, and since their number is distinctly less than the thin leptotene threads, it would appear certain that the double condition is due to the longitudinal association in pairs of the leptotene threads and not to their splitting (Fig. 26).

In the following stages, Figs. 27, 28, shortening and thickening of the paired threads take place, and a definite number of chromosomes can be counted; their number is diploid, and in the latest of these stages many are clearly defined, separate univalents, while others are acrosyndetically paired, being joined at one end by a fine thread. In some cases the pairs are bent round from the point of union so far that the components are parallel to each other (Fig. 28).

In favourable material the fourteen clearly defined chromosomes at this stage are seen to be double, and as their number is diploid, not haploid, the individual leptotene threads which pair in their formation represent monads or half-chromosomes, not whole ones.

True parasynopsis or lateral pairing of whole chromosomes is not seen, only bivalents formed by the end-to-end union of univalents being observed.

In the heterotype division most of the chromosomes are divided, or show the homotypic split in early anaphase, but 1 or 2 undivided univalents are frequently seen at the poles of the spindle; a few 'lagers' are commonly present in the equatorial zone at this stage (Figs. 35, 36).

6. *A. caudata* ♀ × *A. triaristata* ♂.  
( $n = 7$ )                      ( $n = 14$ )

The parents are very distinct species belonging to different sections of the genus *Aegilops*.

At the heterotype metaphase 3-6 acrosyndetic bivalents are present in the normal equatorial position with the univalents, 9-15 in number, scattered irregularly, some of them off the spindle near the poles (Figs. 46, 47).

In early prophase a large number of thin, single threads fill the

nuclear cavity (Fig. 37), condensation and pairing of threads following as in Figs. 38-40.

In the late pachytene stage (Fig. 41) the nuclear membrane is still visible with the nucleolus and a number of discontinuous, thick spireme segments, which continue to contract into clearly defined chromosomes, 21 in number, as in Fig. 42, at which stage bivalents of the end-to-end type are frequently observed. Contraction of the nuclear contents into a dense synizetic knot now occurs, the nuclear membrane and nucleolus remaining visible (Fig. 43). Later, the nuclear membrane and nucleolus disappear and clearly formed chromosomes, paired and unpaired, open out from the contracted knot, the bivalents taking their place on the equatorial plate with the unpaired univalents scattered irregularly as previously noted (Figs. 44-7).

7. *Aegilops caudata* ♀ × *Triticum durum* ♂ (Macaroni Wheat).

( $n = 7$ )

( $n = 14$ )

The gametic numbers of the parents of this hybrid are the same as those of the preceding cross; the plants, however, belong to different genera, the male parent being macaroni wheat, a member of the emmer group.

In the heterotype metaphase from 2-5 acrosyndetic bivalents are present, arranged normally in the equatorial position, the complement of 11-17 univalents being scattered irregularly within the cytoplasm (Figs. 57, 58).

Both in this and the preceding hybrid the univalents are very distinctly shorter and plumper than univalents in any of the other crosses examined.

In early prophase fine leptotene threads fill the nuclear cavity (Fig. 48); later they become associated laterally in pairs and contract into a dense synizetic knot, from which a reduced number of thicker double threads open out, these in turn condensing and contracting into clearly defined chromosomes, diploid in number (Figs. 49-51). The chromosomes frequently reveal their double structure (Fig. a, 51), and some of them are connected end to end in pairs forming typical acrosyndetic bivalents (Fig. 52).

At this stage the nuclear membrane and nucleolus are still present; later these disappear and the chromosomes collect into a dense knot (Figs. 53, 54) from which the univalents and bivalents open out and take their place on the spindle which forms, the metaphase of the division as indicated above being gradually established (Figs. 55-8).

At the heterotype anaphase most of the chromosomes are homotypically divided or split, though a few reach the poles undivided (Fig. 59). The movement of the chromosomes is also irregular at this stage, the number reaching opposite poles varying considerably, and 'lagers' are common.

8. *Aegilops caudata* ♀ × *Triticum vulgare* ♂ (Bread Wheat).  
( $n = 7$ ) ( $n = 21$ )

Like the preceding hybrid this is a cross between *A. caudata* and a cultivated wheat; the latter, however, is Bread Wheat, a hexaploid race with the haploid chromosome number twenty-one, whereas, in the former cross, Macaroni Wheat, a tetraploid race, with haploid number fourteen, was used.

At metaphase there are 14 univalents and 7 bivalents, of which latter 5-6 are acrosyndetic, long and thin, with 1 or 2 of the broken ring 'parasyndetic' type (Figs. 64-6). As usual, the bivalents occupy the normal equatorial position, the univalents being scattered irregularly over the spindle or off it in the cytoplasm.

Early prophase showed a very extensive system of unsplit leptotene threads. Association of the latter in pairs was missed, the next stage observed being apparently late pachytene (Fig. 60), in which the chromosomes are being differentiated, some appearing in pairs with the components connected end to end by a thin thread.

In the succeeding stage (Fig. 61) the chromosomes, presumably the diploid number, are clearly defined within the cell, from which the nuclear membrane and nucleolus have disappeared. The chromosomes now collect into a dense synizetic knot (Fig. 62), out of which the bivalents and univalents unfold and take the metaphase position on the spindle which forms at this time.

At the heterotype anaphase, as is usual in all these hybrids, most of the chromosomes are homotypically divided or split, only a few, which are off the spindle or have collected early at the poles, being undivided. In the equatorial zone at this stage there sometimes appear 'tetrads' as in Fig. 67; these are, however, only end views of the two U-shaped halves of a bent univalent and not the ends of a homotypically split 'parasyndetic' bivalent, which the figure greatly resembles.

9. *Aegilops triaristata* ♀ × *Triticum vulgare* ♂.  
( $n = 14$ ) ( $n = 21$ )

10. *Aegilops triaristata* ♀ × *Triticum vulgare* ♂.  
( $n = 21$ ) ( $n = 21$ )

11. *Aegilops triaristata* ♀ × *Triticum durum* ♂.  
( $n = 21$ ) ( $n = 14$ ).

In the hybrids No. 9 and No. 10 the hexaploid *T. vulgare* is crossed respectively with a tetraploid and hexaploid form of *A. triaristata*.

In the heterotype metaphase of both hybrids, univalents and bivalents are found, the latter always of the acrosyndetic type; in hybrid No. 9 the

number of bivalents varies from one to five, while in No. 10 the number varies from one to seven (Figs. 68-72).

In the hybrid No. 11 the hexaploid form of *A. triaristata* is crossed with the tetraploid *T. durum*; here, from 4-7 bivalents are observed at heterotype metaphase, all of the acrosyndetic type (Fig. 74).

Most of the chromosomes divide homotypically during the anaphase, seven usually finding their way to the poles of the spindle before the rest, which lag for a time in the equatorial zone (Figs. 73, 76). A similar lagging of chromosomes occurs in the second or homotypic division as in Fig. 77.

#### DISCUSSION.

At the heterotype metaphase the chromosomes are so clearly defined that their form and number can be recognized with certainty. In the hybrids described here, and in a previous communication,<sup>1</sup> the chromosomes at this stage are either (1) all paired, only bivalents being found, (2) all separate, unpaired, or (3) both univalents and bivalents present. Counting bivalents as two chromosomes, these with the univalents always equal the total gametic number of the two parents used in the cross.

The form of the bivalents and the manner in which the two components of each are united at this stage of meiosis is also clearly recognized. In some cases the two chromosomes are joined end to end, the pair being arranged in a straight line at right angles to the equatorial plate; in others the bivalent takes the form of a ring or link, with the two chromosomes united at both ends, the association being sometimes so close that the open space within the link is obliterated and the chromosomes lie side by side.

The constancy of these differences in the form of the bivalents, as seen at the heterotype metaphase, suggests that they are of fundamental significance.

As noted elsewhere,<sup>1</sup> the view was expressed that the link-form and the closely arranged parallel association of the two chromosomes of a bivalent is indicative of their exact homology and, where all the chromosomes are thus paired, the relationship of the two parents of a hybrid is as close taxonomically as that between individuals or varieties of the same species. In support of this view may be given the results of the investigation of hybrid No. 1, for at the heterotype metaphase all or most of the bivalents are of the ring type (Figs. 6, 7) and *Triticum monococcum*, one of the parents, is classified as a cultivated derivative of the other parent, *T. aegiloides*.

In the other wheat hybrid, No. 2, *T. Timopheevi* × *T. aegiloides*, seven bivalents are found at metaphase, all the gametic chromosomes of the haploid parent finding mates, the bivalents being chiefly of the parasyndetic type.

<sup>1</sup> See footnote, p. 479.

It was concluded that the end-to-end union of the two chromosomes, in a typical straight acrosyndetic bivalent, was indicative of a more remote relationship or homology, and that the parents of a cross in which this type of bivalent appears at the heterotype metaphase would be classed by the taxonomist as widely different varieties or sub-species. On this hypothesis the relationship between *Aegilops uniaristata* and *A. umbellulata*, parents of hybrid No. 5, in which all the bivalents are acrosyndetic (Figs. 31-3), is more remote than that between *A. uniaristata* and *A. Heldreichii*, parents of hybrid No. 4, in which both the acrosyndetic and the link-form of bivalent are present (Figs. 20-2). This is in accordance with the views of taxonomists, for the two former parents belong to different sections of the genus, while *A. uniaristata* and *A. Heldreichii* have several morphological characters in common, and are placed in the same section of the genus *Aegilops*.

In the hybrids of widely different species, such as *A. ventricosa*  $\times$  *T. dicoccum*, *T. turgidum* and *T. polonicum*, described in a previous communication,<sup>1</sup> all the chromosomes are unpaired at the heterotype metaphase. In hybrid No. 6, *A. caudata*  $\times$  *A. triaristata*, in which the parents are morphologically distinct, and belong to different sections of the genus, the majority of the chromosomes observed at metaphase are unpaired and the bivalents are of the acrosyndetic type.

Similarly, in the wide crosses *A. caudata*  $\times$  *T. durum* and *T. vulgare*, and *A. triaristata*  $\times$  *T. vulgare*, all the bivalents are of the end-to-end type, and accompanied by a number of unpaired chromosomes.

As already noted, the chromosomes are quite clearly defined at the heterotype metaphase, both the form and number of the univalents and the bivalents being recognized with certainty. Moreover, at this stage there is no doubt concerning the limits of the chromosomes forming the bivalents, or the manner in which the syndetic mates are associated. The history, however, of the univalents and bivalents in the earlier stages of prophase is very far from being decisively settled, notwithstanding half a century of cytological research and a plethora of diagrams.

Want of definite knowledge on these matters is, in some degree, due to neglect of phases earlier than diakinesis, but some of the obscurity surrounding those early meiotic stages is to be attributed to the inherent difficulties connected with technique, such as fixation and staining of the nuclear contents at this period of their development. At the same time, it is difficult to get rid of the feeling that correct interpretation of what is observed has not been made easier by the frequent mixing up of cytology and genetics.

In the hybrids investigated in which the early stages of the heterotype division were observed, the metaphase always develops from a dense

<sup>1</sup> See footnote, p. 479.

synizetic knot (Figs. 30, 53, 54, 62). In the stage preceding this, corresponding probably to early diakinesis, and illustrated in Figs. 28, 42, 51, 52, 61, both univalents and bivalents are clearly differentiated. The single chromosomes not infrequently reveal their double nature (Fig. *a* 51), and differ only slightly in regularity of outline and density of staining power from the metaphase univalents. Bivalents when present are always of the end-to-end type, the two component chromosomes of each being either arranged in a straight line or bent round at the point of union at a greater or lesser angle which in some instances reaches 180 degrees when the units become parallel (Figs. *a* 28, 52). The total number of the chromosomes can be counted with certainty, and always equals the sum of the gametic chromosomes of the two parents.

The stage at which syndesis, or pairing of whole chromosomes to form bivalents takes place, and their mode of union, lie within the early prophase, where the exact limits of the structures to which the term chromosome can be applied are not clearly defined.

In some of the hybrids, and in some of those discussed in a previous communication,<sup>1</sup> the nuclear reticulum is resolved into a fine spireme of discontinuous, leptotene threads, undoubtedly single, but their number, unfortunately, cannot be determined, on account of their tenuity and tortuous outline (Figs. 25, 37, 48). Shortly after this, the zygotene spireme appears, consisting of fine threads which are double, a condition which, it is concluded, arises from close parallel association of pairs of single leptotene threads, and not from longitudinal splitting of the latter, for, although their number cannot be definitely counted, the double threads are obviously less numerous than the single threads, and not more numerous, which would be the case if splitting of the latter had taken place (Fig. 26).

The facts just mentioned, and revealed in the examination of the early prophase in some of the hybrids investigated, are now almost universally recognized, but the real nature of the associating leptotene threads is still a matter of controversy: while some look upon them as representing whole chromosomes, and their association as syndesis, resulting in the formation of bivalents (true parasyndesis), others consider them half-chromosomes or monads, their union leading to the formation of univalents or whole chromosomes. The evidence obtained in the researches here described points to the latter view as the correct one in these hybrids.

In hybrid No. 5 the diploid number of chromosomes is fourteen, seven being the gametic number of each parent.

Parallel association of leptotene threads occurs in the early prophase, and a zygotene spireme is produced (Figs. 25, 26). Condensation and segmentation of the spireme takes place later, and from the fact that the number of chromosomes then plainly differentiated is the diploid number,

<sup>1</sup> See footnote, p. 479.

it is clear that the associating leptotene threads represent monads and not whole gametic chromosomes.

Examination of the bivalents found in this hybrid shows that syndesis is always of the end-to-end type; at the heterotype metaphase they are of the straight form (Fig. 31), while earlier their components form an angle with each other, and may become bent round so as to lie parallel (Fig. a 28).

In hybrid No. 1, in which the bivalents are of the ring or slightly open ring type, the conclusion is reached that here, also, pairing of whole chromosomes is acrosyndetic. The association of leptotene threads in parallel pairs is exactly similar to that in the hybrids just mentioned (Figs. 1, 2). Later, however, the thicker, contracted double threads are bent round into loops (apparently seven in number), the free ends of which are connected with the nucleolus (Fig. 3). These loops appear to be the predecessors of the seven ring bivalents, which emerge later from the dense synizetic knot (Figs. 4, 5), and take their place on the equatorial plate at metaphase. On this hypothesis the looped threads represent a pair of chromosomes joined end to end, and bent round until the free ends meet at their point of contact with the nucleolus, the split in the threads of the loops being interpreted as an association of leptotene monads when the zygotene spireme is formed.

It is not possible here to discuss and compare meiosis in these hybrids with that of many other plants in which acrosyndesis is said to prevail. It would appear, however, that the cytological evidence hitherto provided shows that syndesis in *Oenothera* is of the end-to-end type. In this plant seven bivalents are seen at the heterotype metaphase, and the fourteen chromosomes of which they are composed are clearly defined and their limits readily recognized. Some of the bivalents are of the typical acrosyndetic type, the individual chromosomes forming them being joined end to end in a straight line; in the other bivalents the chromosomes are bent and joined at both ends into the form of a ring. Similarly at diakinesis, the diploid number of chromosomes is observed, some of them united at both ends in pairs, others joined at one end only, or end to end in chains of variable length, the segmentation of these chains into pairs providing the straight acrosyndetic bivalents observed at metaphase.

The fine connexions between one chromosome and the next often appear double, and indications of the double nature of the fourteen univalents so connected are also sometimes visible. If the longitudinal halves of these chromosomes, thus indicated, correspond to the leptotene threads which come together at the zygotene stage, as seems likely, it is clear that such threads are not whole chromosomes, but halves or monads, and their association should not be termed parasyndesis.

In hybrids of widely different species of *Aegilops*  $\times$  wheat crosses such as *A. ventricosa*  $\times$  *T. turgidum*, *T. dicoccum*, or *polonicum*, only univalents

are found at the heterotype metaphase; investigation of the prophases of these should prove of especial interest in this connexion, for if the parallel association of leptotene threads should be discovered, the double structures so produced could hardly be interpreted as true parasyndetic bivalents, unless their reduction to univalents takes place before the heterotype metaphase is reached.

#### SUMMARY.

1. Meiosis was investigated in the pollen mother-cells of eleven hybrids between species of wheat and *Aegilops* of different degrees of taxonomic relationship.

2. The chromosomes of the heterotype metaphase, in these hybrids, always open out from a dense synizetic knot, which is formed immediately after a stage (diakinesis?) in which all the chromosomes are clearly defined.

3. At the heterotype metaphase varying numbers of univalents and bivalents are observed: the latter are of two types, namely, (1) the typical acrosyndetic form in which the two component chromosomes are joined end to end in a straight line, and, (2) the parasyndetic type in which the chromosomes are united in the form of a ring or broken link. Chromosomes uniting in the latter manner are considered to be exactly homologous, while those joined end to end, as in the acrosyndetic bivalents, are more remotely related.

4. In the hybrids between different species or widely different varieties of a species, all or most of the chromosomes are homotypically divided or split at anaphase of the heterotype division, and 'lagging' chromosomes are always observed in the anaphase of this division as well as in the succeeding homotypic division, even when the gametic number of chromosomes is the same in both parents.

5. The single leptotene threads of the early prophase are considered to be half-chromosomes, or monads, their association leading to the formation of whole chromosomes (univalents), not bivalents.

6. Both the straight acrosyndetic and the ring parasyndetic bivalents appear to be formed in the same manner, namely, by the end-to-end union of whole chromosomes, which in the parasyndetic type are bent round through an angle of 180 degrees, sometimes becoming joined at both ends.

7. In some of the hybrids all the bivalents are of the same type, in others both types are found, while in a third group only univalents are seen at the heterotype metaphase.

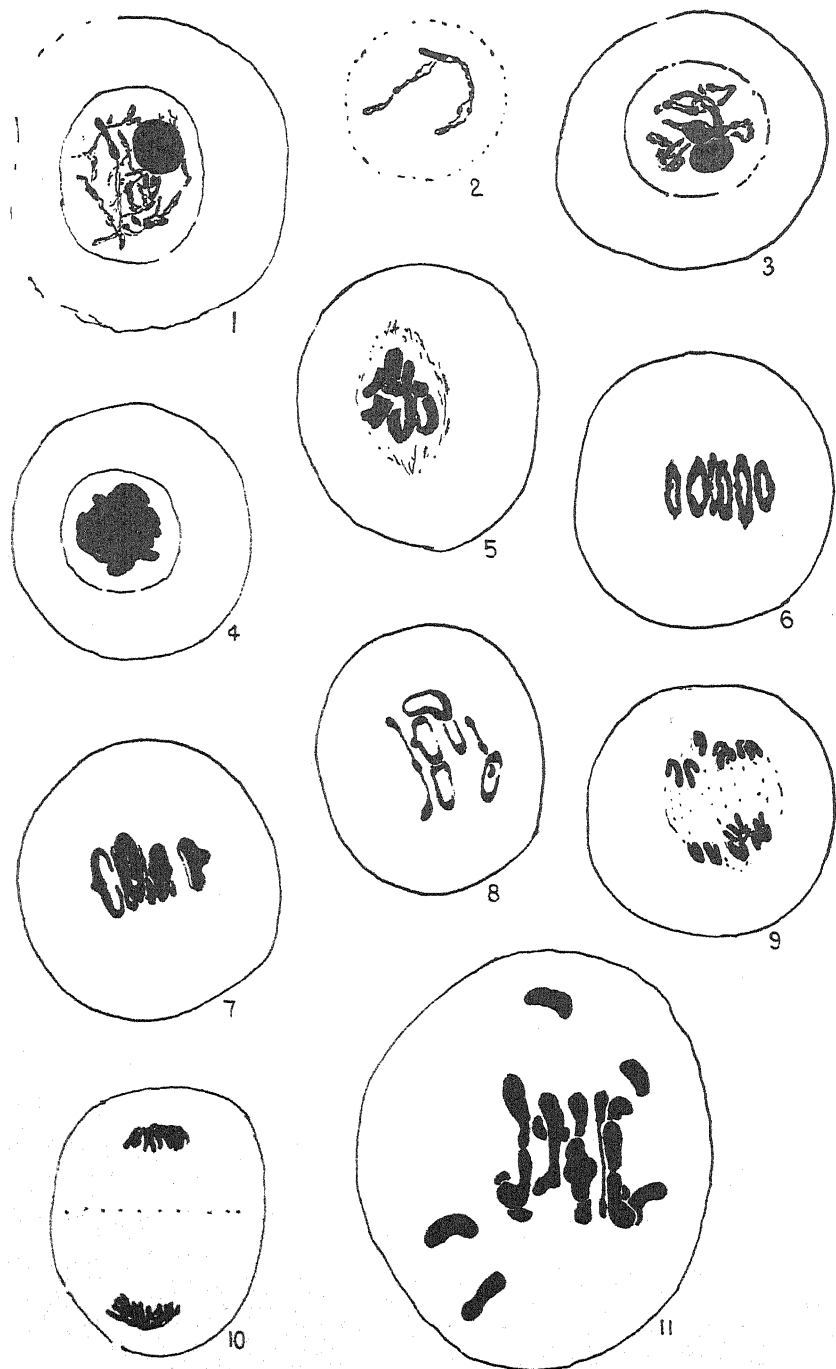
The results of these investigations suggest that (a) where all the bivalents are of the 'parasyndetic' or ring type, the parents of such a cross belong to the same species; (b) where only acrosyndetic bivalents are found, the parents of the hybrids are more remotely related, being usually more marked varieties or sub-species, (c) where only univalents are observed



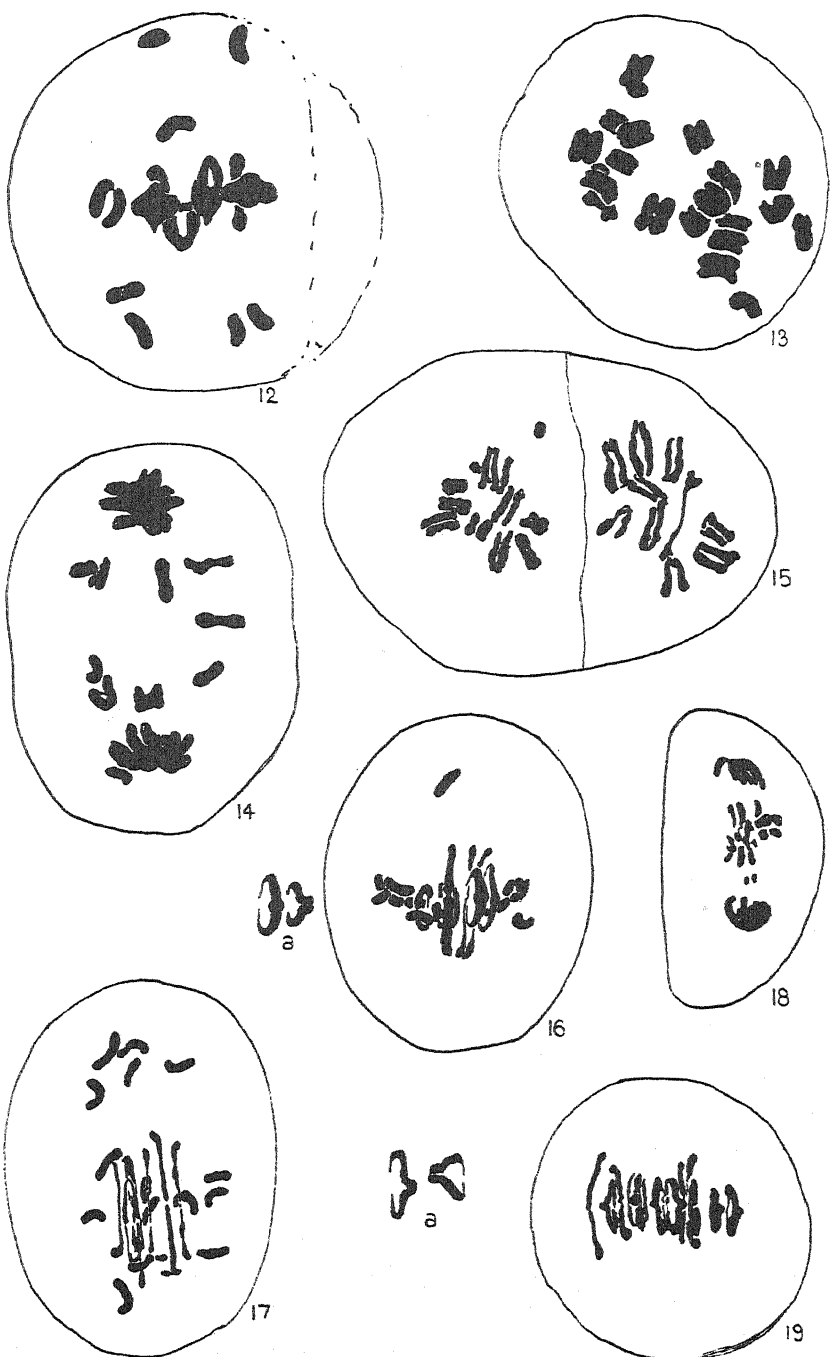
at metaphase the parents of the hybrids are still more widely separated, belonging to different species.

Determination of the proportion of univalents to bivalents and the type of the latter, in hybrids between two plants, may ultimately assist the taxonomist to settle difficult questions of relationship.

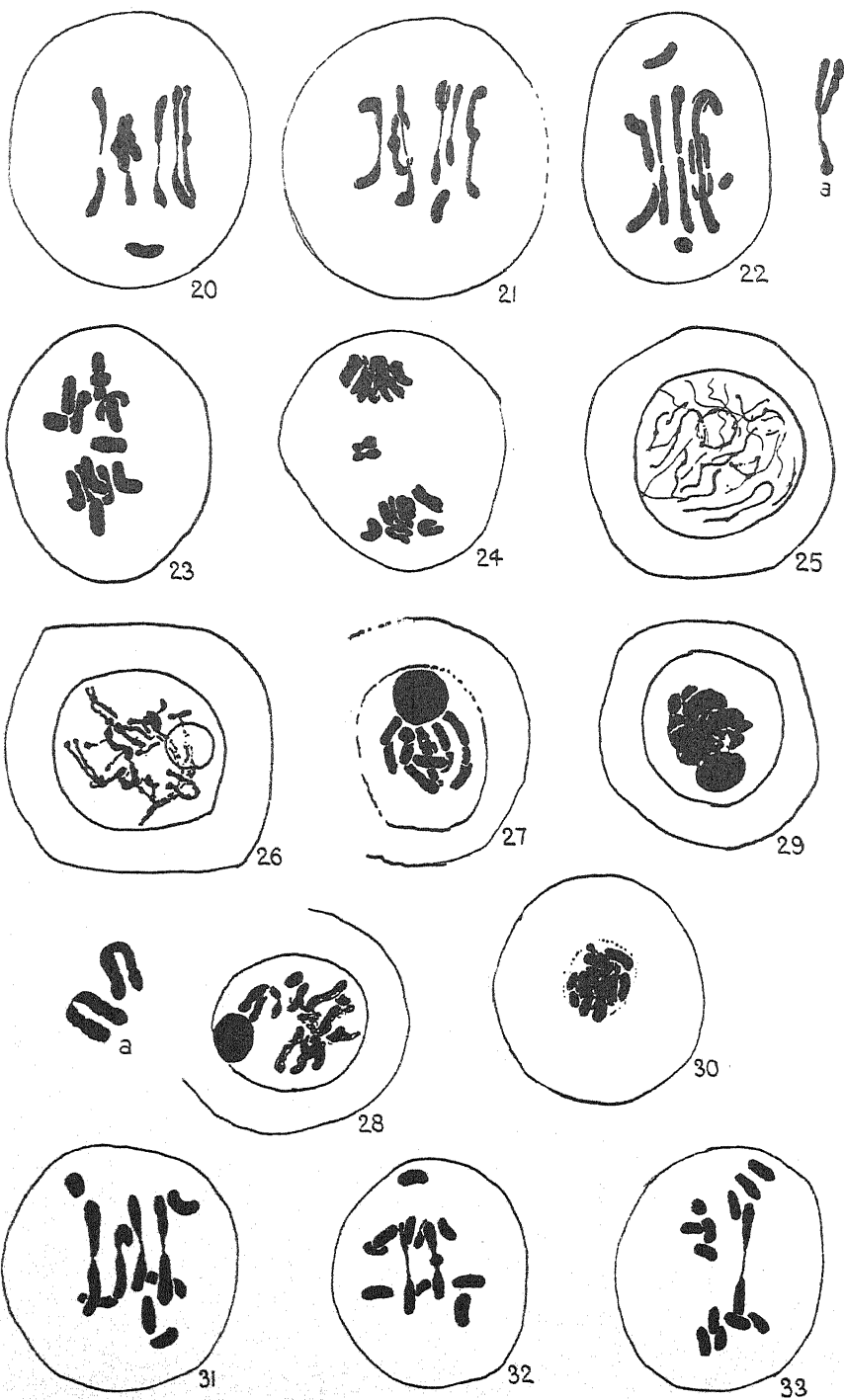
I desire to express my thanks to the Research Board of the University for the grant from the Huntley and Palmer Research Fund, and to Miss Pantin, B.Sc., for devoted assistance in the work of hybridization and the preparation of the sections involved in these investigations.



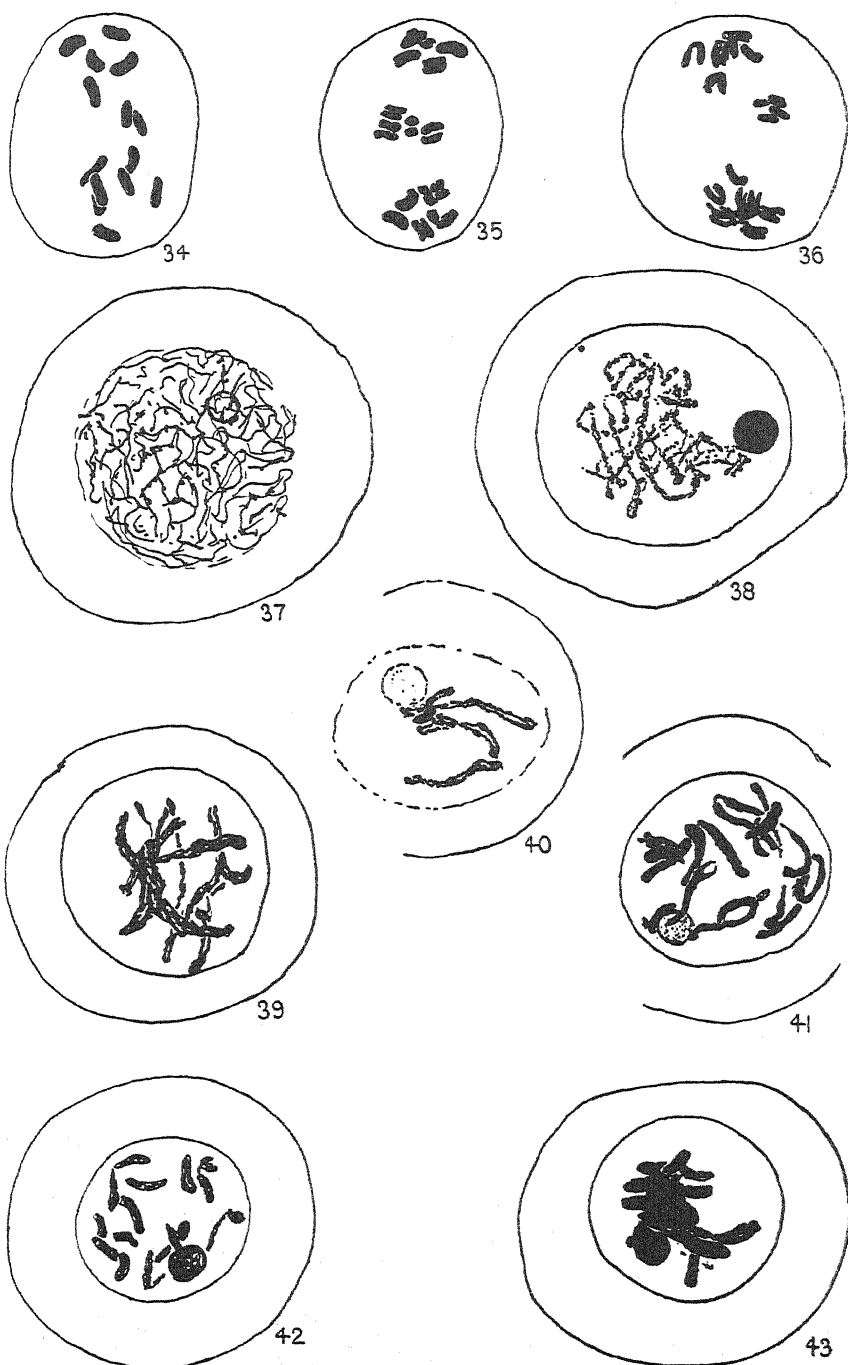
FIGS. 1-10.  $F_1$  of *Triticum monococcum*  $\times$  *T. aegilopoides*.  
 FIG. 11.  $F_1$  of *Triticum dicoccum* var. *Timopheevi*  $\times$  *T. aegilopoides*.



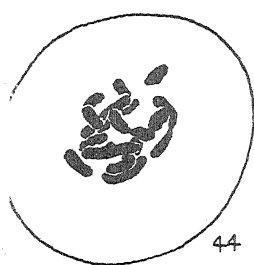
FIGS. 12-15.  $F_1$  of *Triticum dicoccum* var. *Timopheevi*  $\times$  *T. aegilopoides*.  
 FIGS. 16-18.  $F_1$  of *Aegilops ovata*  $\times$  *A. triaristata* ( $n = 14$ ).  
 FIG. 19. *A. ovata*.



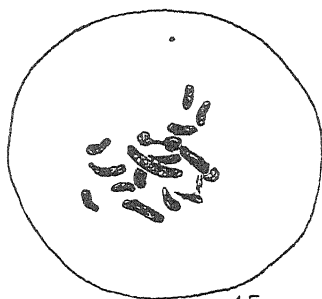
FIGS. 20-24.  $F_1$  of *Aegilops uniaristata*  $\times$  *A. Heldreichii*.  
 FIGS. 25-33.  $F_1$  of *A. uniaristata*  $\times$  *A. umbellulata*.



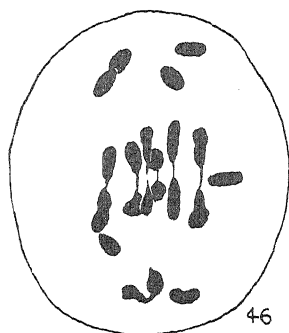
FIGS. 34-6.  $F_1$  of *Aegilops uniariolata*  $\times$  *A. umbellulata*.  
 FIGS. 37-43.  $F_1$  of *A. caudata*  $\times$  *A. triaristata* ( $n = 14$ ).



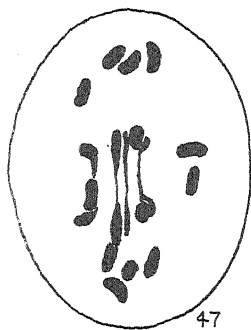
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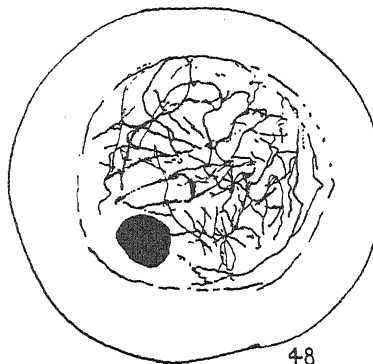
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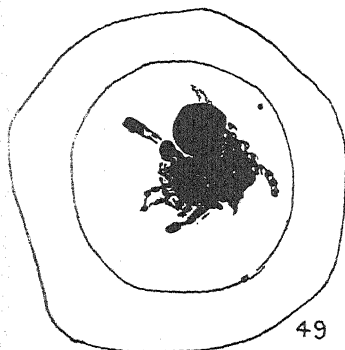
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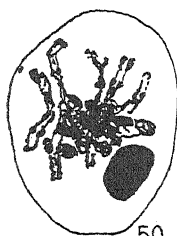
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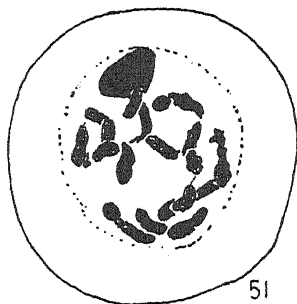
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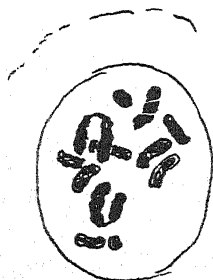
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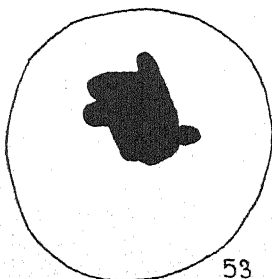
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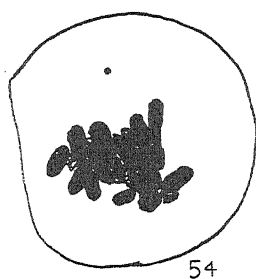


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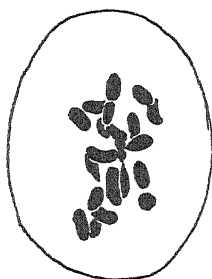


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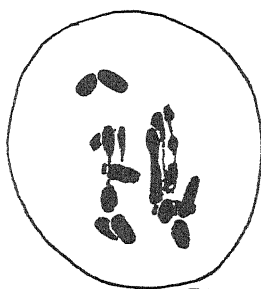
FIGS. 44-7.  $F_1$  of *Aegilops caudata*  $\times$  *A. triaristata* ( $n = 14$ ).  
FIGS. 48-53.  $F_1$  of *A. caudata*  $\times$  *Triticum durum*.



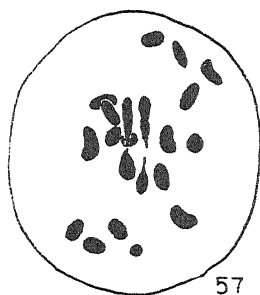
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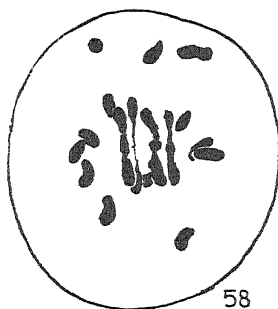
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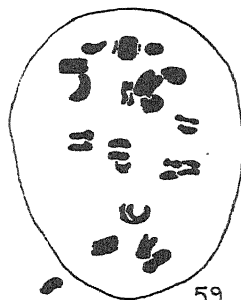
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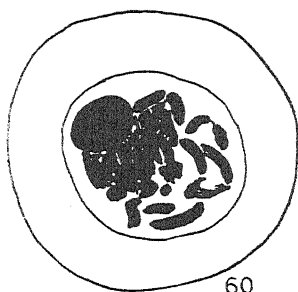
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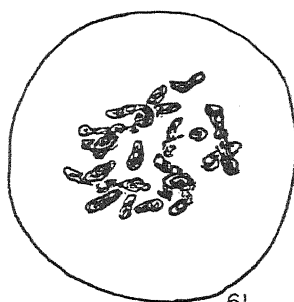
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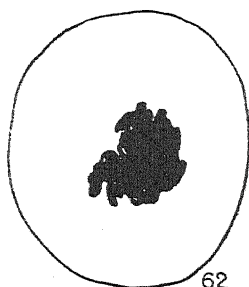
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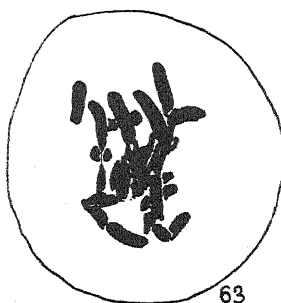
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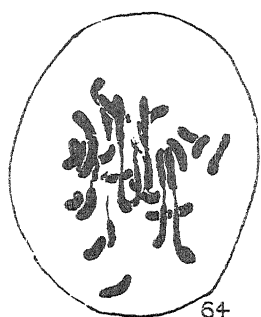


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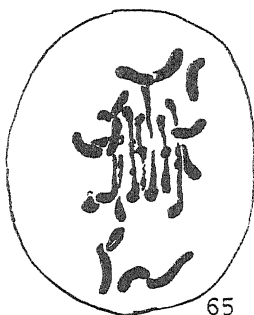


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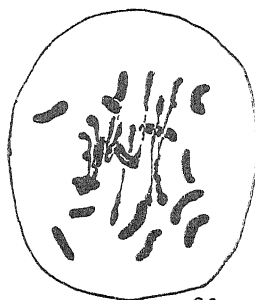
FIGS. 54-9.  $F_1$  of *Aegilops caudata*  $\times$  *Triticum durum*.  
FIGS. 60-3.  $F_1$  of *A. caudata*  $\times$  *Triticum vulgare*.



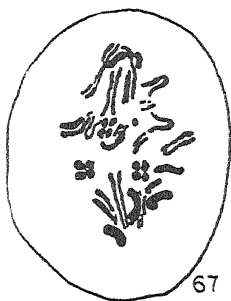
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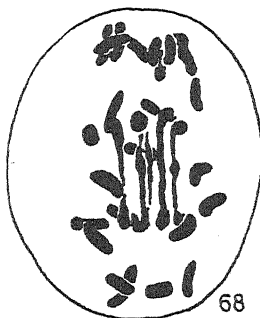
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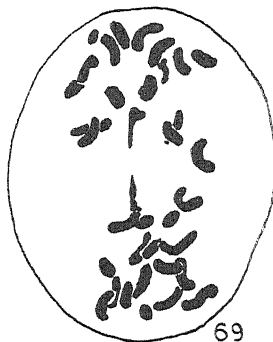
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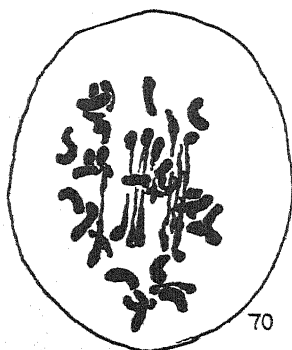
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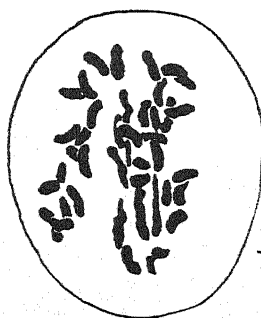
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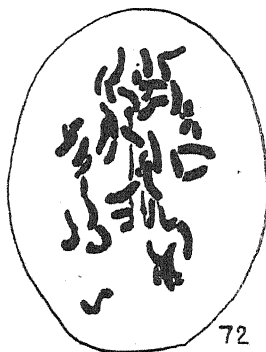
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FIGS. 64-7.  $F_1$  of *Aegilops caudata*  $\times$  *Triticum vulgare*.  
FIGS. 68, 69.  $F_1$  of *A. triaristata* ( $n = 14$ ), *Triticum vulgare*.  
FIGS. 70, 71.  $F_1$  of *A. triaristata* ( $n = 21$ ),  $\times$  *T. vulgare*.



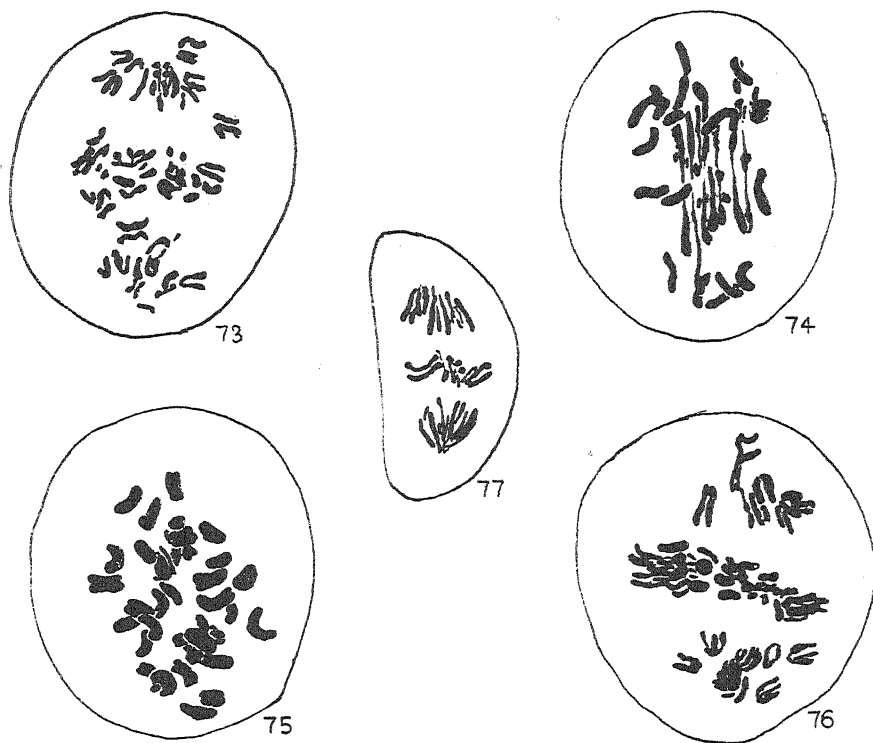


FIG. 73.  $F_1$  of *A. triaristata* ( $n = 21$ )  $\times$  *T. vulgare*.

FIGS. 74-7.  $F_1$  of *A. triaristata* ( $n = 21$ )  $\times$  *T. durum*.

## EXPLANATION OF FIGURES.

All the figures are drawn with the aid of Abbe's camera lucida, and are from single sections of permanent preparations except Figs. 11-15, which are from fresh material stained with acetocarmine. Zeiss apochromat, 2 mm. objective and No. 12 ocular.

Fig. 1-10.  $F_1$  of *Triticum monococcum*  $\times$  *T. aegilopoides*.

- Fig. 1. Discontinuous zygotene spireme.
- Fig. 2. Two separate threads of zygotene spireme.
- Fig. 3. Pachytene loops (7?).
- Fig. 4. Dense synizetic knot.
- Fig. 5. Formation of spindle and opening out of Fig. 4.
- Fig. 6. Heterotype metaphase with 7 ring bivalents.
- Fig. 7. Heterotype metaphase with 7 ring bivalents.
- Fig. 8. Heterotype metaphase, bivalents displaced.
- Fig. 9. Heterotype anaphase.
- Fig. 10. Heterotype telophase.

Figs. 11-15.  $F_1$  of *Triticum dicoccum*, var. *Timopheevi*  $\times$  *T. aegilopoides*.

Figs. 11-12. Heterotype metaphases.

Fig. 13. Beginning of heterotype anaphase; most of the chromosomes heterotypically divided or split.

Fig. 14. Heterotype anaphase with lagging chromosomes and monads.

Fig. 15. Polar view of homotypic metaphase; 1 cell with 10 divided chromosomes, the other with 10 divided and 1 undivided chromosomes.

Figs. 16-18.  $F_1$  of *Aegilops ovata*  $\times$  *A. triaristata* ( $n = 14$ ).

Fig. 16. Heterotype metaphase; 7 bivalents (4 parasynthetic, 3 acrosynthetic); at  $\alpha$  are 2 parasynthetic bivalents.

Fig. 17. Heterotype metaphase; 6 (? 7) bivalents.

Fig. 18. Homotype anaphase showing lagging chromosomes.

Fig. 19. *A. ovata*.

Fig. 19. Heterotype metaphase; 14 bivalents; at  $\alpha$ , 2 parasynthetic bivalents.

Figs. 20-4.  $F_1$  of *Aegilops uniaristata*  $\times$  *A. Heldreichii*.

Fig. 20. Heterotype metaphase; 6 bivalents (5 parasynthetic, 1 acrosynthetic), and 2 univalents.

Fig. 21. Heterotype metaphase; 6 bivalents, 2 univalents.

Fig. 22. Heterotype metaphase; 5 bivalents, 4 univalents; at  $\alpha$  a Y-shaped trivalent.

Fig. 23. Beginning of heterotype anaphase.

Fig. 24. Late heterotype anaphase.

Figs. 25-36.  $F_1$  of *Aegilops uniaristata*  $\times$  *A. umbellulata*.

Fig. 25. Leptonema.

Fig. 26. Zygotene spireme.

Fig. 27. Late pachytene.

Fig. 28. Near diakinesis; at  $\alpha$  2 acrosynthetic bivalents, the components bent round into a parallel position.

Fig. 29. Synizesis following Fig. 28.

Fig. 30. Late stage of Fig. 29.

Fig. 31. Heterotype metaphase; 4 acrosynthetic bivalents, 6 univalents.

Fig. 32. Heterotype metaphase; 2 acrosynthetic bivalents, 10 univalents.

Fig. 33. Heterotype metaphase; 1 acrosynthetic bivalent, 12 univalents.

Fig. 34. Heterotype metaphase; 14 univalents.

Fig. 35. Heterotype anaphase; 4 lagging, divided univalents, the monads (8) clearly separate.

Fig. 36. Late heterotype anaphase.

Figs. 37-47.  $F_1$  of *Aegilops caudata*  $\times$  *A. triaristata* ( $n = 14$ ).

Fig. 37. Leptonema.

Fig. 38. Zygotene spireme.

Fig. 39. Late zygotene spireme.

Fig. 40. Separate zygotene threads.

Fig. 41. Segmented pachytene spireme, bivalents appearing.

Fig. 42. Near diakinesis.

Fig. 43. Synizesis following Fig. 42.

Figs. 44-5. Beginning of heterotype metaphase.

Fig. 46. Heterotype metaphase; 7 acrosynthetic bivalents, 7 univalents.

Fig. 47. Heterotype metaphase; 4 acrosynthetic bivalents, 13 univalents.

Figs. 48-59.  $F_1$  of *Aegilops caudata*  $\times$  *Triticum durum*.

Fig. 48. Leptonema.

Fig. 49. Synizesis.

Fig. 50. Opening out of synizetic knot, Fig. 49.

Fig. 51. Diakinesis (?); 21 chromosomes, some of them united in pairs end to end; at  $\alpha$  two univalents showing double structure.

Fig. 52. Diakinesis (?); part of nucleus with 2 acrosynthetic bivalents, the components bent round into parallel position.

Figs. 53-4. Synizetic knots following Fig. 51.

Fig. 55. Beginning of heterotype metaphase unfolding from Fig. 53.

Fig. 56. Heterotype metaphase, 5 acrosyndetic bivalents, 11 univalents.

Fig. 57. Heterotype metaphase, 2 acrosyndetic bivalents, 17 univalents.

Fig. 58. Heterotype metaphase, 4 acrosyndetic bivalents, 13 univalents.

Fig. 59. Heterotype anaphase showing split, divided, and undivided univalents.

Figs. 60-7.  $F_1$  of *Aegilops caudata*  $\times$  *Triticum vulgare*.

Fig. 60. Near diakinesis.

Fig. 61. Diakinesis (?).

Fig. 62. Synizetic knot following Fig. 61.

Fig. 63. Opening out of knot of Fig. 62.

Figs. 64-6. Heterotype metaphases; 7 acrosyndetic bivalents, 14 univalents.

Fig. 67. Heterotype anaphase, showing false tetrads—the end views of homotypically divided, bent univalents.

Figs. 68-9.  $F_1$  of *Aegilops triaristata* ( $n = 14$ )  $\times$  *Triticum vulgare*.

Fig. 68. Heterotype metaphase; 5 acrosyndetic bivalents, 25 univalents.

Fig. 69. Early heterotype anaphase.

Figs. 70-3.  $F_1$  of *Aegilops triaristata* ( $n = 21$ )  $\times$  *Triticum vulgare*.

Fig. 70. Heterotype metaphase; 7 acrosyndetic bivalents, 28 univalents.

Fig. 71. Heterotype metaphase; 3 acrosyndetic bivalents, 36 univalents.

Fig. 72. Heterotype metaphase; 3 acrosyndetic bivalents, 36 univalents.

Fig. 73. Heterotype anaphase, all chromosomes homotypically divided.

Figs. 74-7.  $F_1$  of *Aegilops triaristata* ( $n = 21$ )  $\times$  *Triticum durum*.

Fig. 74. Heterotype metaphase; 7 acrosyndetic bivalents, 21 univalents.

Fig. 75. Beginning of heterotype anaphase; many chromosomes showing the homotypic split.

Fig. 76. Heterotype anaphase; most chromosomes homotypically divided. The components of the seven reduced bivalents have moved to the two poles and are divided homotypically; most of the 21 univalents have also divided and are found lagging in the equatorial zone.

Fig. 77. Anaphase of the homotypic division showing lagging chromosomes.



# A Cretaceous Gleicheniaceus Fern from Western Greenland.

BY

T. G. TUTIN.

(Botany School, Cambridge.)

Plate XVI and two Figures in the Text.

WHILE engaged in examining a collection of Cretaceous fossil plants from West Greenland, made by Dr. Hartz in 1890 and lent by the Mineralogical Museum of Copenhagen to Professor Seward, portions of small Gleicheniaceus fronds were found. These differ considerably from most species of *Gleichenites* in having 20 to 40 sporangia in the sorus, and the sori very crowded on the pinnules. On account of these and other differences to be described later, it seems best to separate this fern, and a few other species, from *Gleichenites*: a new genus *Gleicheniopsis* is proposed for them.

*Gleicheniopsis*. Ferns with small pinnules bearing large, crowded, simple sori with from 10 to 40 small sporangia in each. Spores large, few in each sporangium (about 32). Age, Lower Cretaceous.

## I. *Gleicheniopsis fecunda* comb. nov.

*Aspidium fecundum* Heer (3).

*Polypodium Graahianum* Heer (4).

*Gleichenites Gieseckiana* (Heer) Seward (5) ex parte.

The material examined came from two localities, Ritenbenk's Coal-mine on Disko Island and Patoot on the mainland opposite.

The rock in both cases is a fine dark grey shale. The pinnae occur as small pieces not more than about 3 cm. long, with numerous alternate pinnules 0.25 cm. to 0.3 cm. long and 0.15 cm. to 0.25 cm. wide at the broadest part, with a narrow strip of connecting lamina about 0.05 cm. wide between adjacent pinnules. The appearance of the pinna and the venation of the pinnules is shown in Text-fig. 1. This drawing was made from a specimen from Patoot which showed the upper surface of the pinnules. A transfer preparation<sup>1</sup> made from the Patoot fossil showed that the sori and sporangia correspond very closely with those of the more

<sup>1</sup> Walton (7).

numerous specimens from Ritenbenk's Coal-mine, which, however, do not show the venation well.

The venation of the pinnules is of the usual type found in *Gleichenites* and in living species of *Gleichenia*, the lowest branch being anadromic (arising on the 'lower' side). There are four to five pairs of lateral veins which generally fork once near the margin of the pinnule but are sometimes unbranched. In the smaller pinnules towards the end of the pinna the lowest lateral vein does not fork. Associated with these fronds are numerous dichotomizing axes and a few circinate buds of a Gleicheniaceus type. These were not found in actual connexion with the pinnae, but it is highly probable that they are all parts of the same plant, particularly as no pieces of any other fern occur in association with the *Gleicheniopsis*.

Another specimen from Patoot (Pl. XVI, Fig. 1) shows the attachment of the pinnae to the rachis. This specimen also proves that the broken fragments are not small distal portions of large pinnae, but are the normal size for this fern.

Most of the pinnae from Ritenbenk's Coal-mine are fertile, bearing from five to seven large sori on each pinnule (Text-fig. 2 and Pl. XVI, Fig. 2). The sori are crowded and, while remaining quite distinct from one another, often lose their circular outline through contact with their neighbours. There are from 20 to 40 sporangia in each sorus (Pl. XVI, Fig. 3), and they all appear to mature simultaneously, as the spores within them are all at the same stage of development.

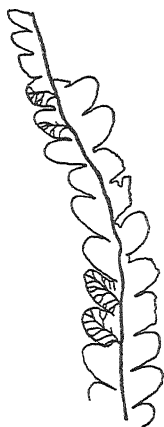
The sporangia are small for a Gleicheniaceus fern, being about 0.17 mm. in diameter with an annulus of about ten remarkably long indurated cells, which is not well preserved. The sporangia were separated by maceration in nitric acid and potassium chlorate, and also by warming in pyridine. This treatment destroys most of the sporangium wall, but leaves masses of spores corresponding in size and shape to the sporangia. The masses of spores, liberated on maceration of the sporangium, vary between 0.15 mm. and 0.17 mm. in diameter and 0.23 mm. and 0.28 mm. in length and appear to have a short thick 'stalk' (Plate XVI, Fig. 4).

On adding ammonia to the sporangial groups the tetrahedral spores separate, and the number of spores in a single sporangium may be counted. The average of counts made with six different sporangia gave just under 22 spores per sporangium, the actual counts varying between 18 and 26. This probably indicates a 'typical' spore number of 32. The spores vary in size between 0.05 mm. and 0.06 mm. Their markings and shape are shown in Pl. XVI, Fig. 5.

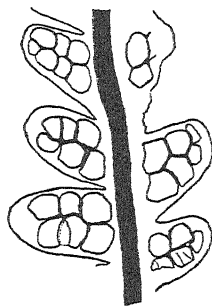
Heer's figures of *Aspidium fecundum* do not show the size and shape of the sori accurately, but his specimens have been refigured by Seward (5), Text-figs. 2 A and B. The 'indusium' shown in Heer's figure is probably the scar left when the sorus was broken off.

II. *Gleicheniopsis Sewardii* sp. nov.*Gleichenites Gieseckiana* (Heer) Seward (5) ex parte.

The type specimen of Heer's *Gleichenia Gieseckiana* (3) has 5 to 6 sporangia in the sorus, while some of the ferns described by Seward under



TEXT-FIG. 1.



TEXT-FIG. 2.

TEXT-FIG. 1. Portion of pinna of *G. fecunda* showing venation and form.  $\times 2$ . Patoot.

TEXT-FIG. 2. Portion of pinna of *G. fecunda* showing sori.  $\times 4$ . Disko Island.

*Gleichenites Gieseckiana* have 12 to 20 sporangia in the sorus. They cannot, therefore, be regarded as belonging to Heer's species. Of the species given in synonymy under *Gleichenites Gieseckiana* (Heer) Seward (5) none can be regarded as undoubtedly identical with the species now under consideration, though this is probably due to the scanty descriptions given, or the lack of sporangia in the specimens.

The type of *Gleicheniopsis Sewardii* is figured by Seward (5), Pl. V, Figs. 9 and 16. Other specimens undoubtedly belonging to this species are shown in Figs. 1 (sterile specimen) and 14 (fertile specimen). The localities are Pagtorfik (type) and Kaersuarsuk.

The new species differs from *G. fecunda* in its larger size, and narrower, more pointed, often falcate pinnules, with a broader webbing between them. Fertile pinnae are rare and the usual number of sori on a pinnule is three. The sori do not touch one another as they do in *G. fecunda*. The number of sporangia in the sorus is lower than in *G. fecunda*, varying between 12 and 20, of about 0.17 mm. in diameter.

No spores have been obtained from any specimens of this fern, but from the size of the sporangium it is probable that the spore number does not exceed that of *G. fecunda*.

III. *Gleicheniopsis* sp.

*Gleichenites Gieseckiana* (Heer) Seward (5) ex parte.

? *Gleichenia gracilis* Heer (4).

A small fern, possibly the same as *G. fecunda* but differing from it in its smaller size and fewer sori, is figured by Seward (5), Pl. V, Figs. 12, 12 A, and Text-fig. 2 C.

There are generally three sori on the pinnule and 20 sporangia in the sorus. The pinnules are relatively broad and taper suddenly to an obtuse apex. They are 0.15 cm. long and 0.1 cm. broad. The locality is Pagtorfik.

In the general form of the frond and method of branching of the rachis *Gleicheniopsis* resembles living *Gleichenias*. In the species of *Gleichenia* with small pinnules about the same size as those in *Gleicheniopsis* there is only one sorus, and species with numerous sori have only one sorus to each group of veins corresponding to the veins of a single pinnule of *Gleicheniopsis*. The whole pinnule can be regarded as equivalent to a number of small pinnules with the webbing between them complete. Thus the sori, when there are several on a pinnule, are always widely spaced in the living Gleicheniaceae, not crowded as in the three fossil species here described.

In *Gleichenia* the number of sporangia in the sorus varies from two in *G. dicarpa* Br. to ten in *G. pectinata* Pr., the sporangia are large, 0.3 to 0.4 mm. in diameter, and the typical spore number varies between 256 in *G. pectinata* Pr. and 1024 in *G. flabellata* Br. In this respect *Gleichenia* is very different from *Gleicheniopsis*, which is remarkable in having a simple sorus with numerous crowded sporangia each containing a few large spores.

The Protocyatheaceae (*Lophosoria* and *Metaxya*) Bower (1) approach these fossils in having a typical spore number of 64, while *Metaxya* has a large flat sorus of 50 to 100 sporangia. The form and branching of the frond is, however, very different, and there appears to be no reason for believing them to be closely related.

In *Gleichenites* sori are known in many cases, but details of the sporangia and spores are lacking, often owing to bad preservation. The few species for which sufficient data are available show a wide range of soral structure. *G. Nordenskiöldii* (Heer) Seward has large sporangia 0.36 mm. long. There are only a few sporangia in each sorus, but the sori are not very clearly delimited, and there are always several on a pinnule (5). From a count made on a rather badly preserved sporangium the typical spore number appears to be about 128. *G. nitida* Harris (2) has only one sorus on each pinnule with from 1 to 12 sporangia 0.22 mm. in diameter, and the spore number is about 100.



*Gleichenites Porsildii* Seward (5) has 6 to 10 sori closely crowded on the fairly large pinnules. The pyriform sporangia, of which there are 40 to 50 in each sorus, contain about 200 small spores. The sporangia are large, being 0.36 mm. long and 0.18 mm. wide (6). Owing to the large number of sporangia in the sorus and the crowding of the sori on the pinnule this species might be placed in *Gleicheniopsis*, but the large spore number and the size of the sporangium agree better with *Gleichenites*.

The distinctive features of *G. Porsildii* should perhaps be regarded as worthy of generic rank in the future, particularly if other species with similar characters are found, but for the present it seems best to leave it in *Gleichenites*.

Thus the Gleicheniaceae, which are considered a primitive family, show a greater range of reproductive structure in the Cretaceous than they do at present. The species of *Gleicheniopsis* show an increase in the number of sporangia in the sorus to such a point that the dehiscence of each sporangium probably interfered with that of its neighbours (1). It may possibly have been this which led to the extinction of these ferns in competition with flowering plants and other ferns with gradate or mixed sori capable of giving a greater spore output.

The following table summarizes the soral characters of the species discussed:

Species.	No. of sporangia in the sorus.	Diam. of sporangia.	No. of spores in sporangium.	Size of spores.	No. of sori per pinnule.	Size of pinnule.
		mm.		mm.		
<i>Gleicheniopsis</i> <i>fecunda</i>	20-40	0.17	22 (32)	0.05-0.06	5-7	small
<i>Gleicheniopsis</i> <i>Sewardii</i>	12-20	0.17	(32 *)	0.05 *	2-4	small
<i>Gleichenites</i> Nor- <i>denskiöldii</i>	2-3	0.3	(128?)	0.03	numerous	small
<i>Gleichenites</i> <i>Porsildii</i>	40-50	0.36	(256?)	0.02 ?	6-10	medium
<i>G. nitida</i>	1-12	0.22	100?	0.028	1	small
<i>Gleichenia</i> <i>flabellata</i>	2-4	0.4	(1024)	0.03	numerous	large
<i>Lophosoria</i> <i>quadripinnata</i>	7-10	0.2	(64)	—	1	small

The numbers in brackets are the typical spore numbers.

\* Estimated.

? Approximate.

## SUMMARY.

1. A new genus *Gleicheniopsis* is proposed for certain species formerly included in *Gleichenites* which differ markedly from *Gleichenia* in having numerous small sporangia in the sorus and a very low spore number.

2. The species described are readily distinguishable when fertile, but are generally impossible to determine definitely when sterile.

3. A comparison is made between the living and fossil members of the Gleicheniaceae.

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  5. SEWARD, A. C. : The Cretaceous Plant-bearing Rocks of Western Greenland. Phil. Trans. B, ccxv. 69, 1926.
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#### EXPLANATION OF PLATE XVI.

Illustrating Mr. T. G. Tutin's paper on A Cretaceous Gleicheniacean Fern from Western Greenland.

Fig. 1. *Gleicheniopsis fecunda* rachis with pinna attached. × 4. Patoot.

Fig. 2. *G. fecunda*. Part of a pinna with sori. × 10. Ritenbenk's Coal-mine, Disko Island.

Fig. 3. *G. fecunda*. One pinnule showing sori and sporangia. × 20. Ritenbenk's Coal-mine, Disko Island.

Fig. 4. *G. fecunda*. Spore-mass liberated on maceration. × 190. Ritenbenk's Coal-mine, Disko Island.

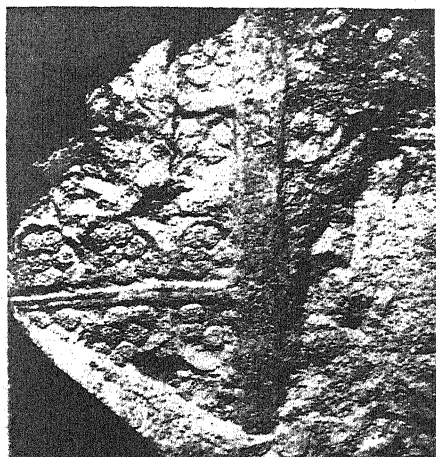
Fig. 5. *G. fecunda*. Spore. × 220. Ritenbenk's Coal-mine, Disko Island.



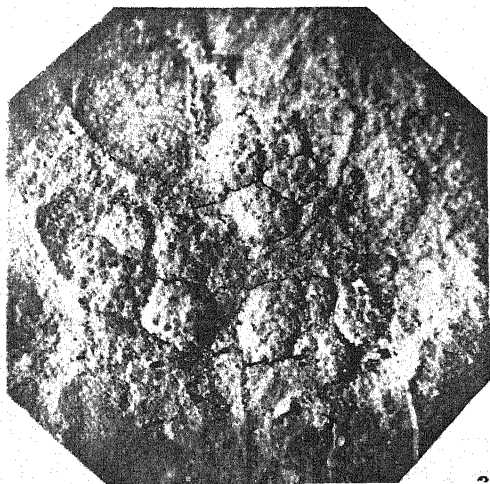
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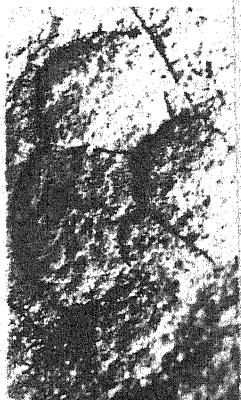
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1



2



3



# Introduction to the General Cytology of the Cruciferae.

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With one hundred and twenty Figures and four Diagrams in the Text.

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## INTRODUCTION.

THIS work was undertaken with the double object of testing the applicability of cytology to the solution of *taxonomic problems* and of obtaining evidence on the relation between *chromosome changes and the natural evolution of species and genera*.

The field was purposely localized by the selection of a single natural family, and the Cruciferae, owing to their abundance of available representatives, appeared to be especially suitable. Within this boundary, however, the aim has been towards a general survey rather than the exhaustive pursuance of problems of detail, which have in the more interesting instances been reserved for separate treatment. The reasons for this course are many.

On the *taxonomic* side the difficulty of detailed classification within a large but rather uniform group gives immediate practical importance to any provision of additional diagnostic characters. On the other hand, the value of cytological data to the systematist cannot be foretold, but will depend on the degree of chromosomal uniformity met with. This will not necessarily be the same in different circles of affinity, and can only be

determined by a general survey. Further, recent cytogenetical literature has multiplied instances of remarkable differences in chromosome number, size, and shape occurring within one taxonomic species, or even one pure line, without corresponding differences in the external 'systematic characters'.<sup>1</sup>

Uncritical application of cytological data may thus be most misleading. Within the Cruciferae, for instance, the cytology has afforded conclusive evidence that '*Capsella procumbens*' should rightly be transferred to *Hutchinsia*, but the same degree of chromosomal difference does not yet warrant the unequivocal inclusion of *Diplotaxis catholica* as a *Brassica*. The general character of the whole assemblage of neighbouring forms rather than individual peculiarities of the species immediately concerned alone discriminates between the two cases. The chief aim and value of the work, therefore, from the systematic standpoint, is in the provision of a wide plane of reference by which the interpretation of individual facts may be assessed. Guidance is also incidentally afforded for the subsequent choice of problems for more intensive study.

On the *evolutionary* side the more natural the family the greater is the chance that trends of change in nuclear, as in gross, morphology may remain discernible in spite of much extinction. One may thus legitimately expect that a general survey should reveal some logical connexion between cytological types indicating evolutionary trends of the past. But that evolution can still occur in the Cruciferae is indicated by the artificial production of a new form of generic status, the *Raphano-Brassica* of Karpechenko (24). That new species have recently arisen is suggested by the simple genetical analysis of *Capsella Viguieri* by Shull (48), and by evidence from geographical distribution in dating some species of *Cardamine* as post-glacial in origin (see Leopold (30)). Any discernible cytological trends should therefore illuminate the rôle played by chromosomal changes in the production of new forms under natural, in comparison with experimental, conditions.

The general conclusions are presented separately (see pp. 532-9) from the detailed consideration of the full cytological data (p. 513 et seq.), which is summarized by diagrams (I-IV pp. 533-7). The complete list of chromosome numbers is put with the summary at the end.

Whilst the work has been in progress two publications by Jaretsky (21 and 22) have appeared independently on the same subject. His results have been incorporated and, where necessary, criticized. Duplication of his work has been entirely fortuitous, but since he has concentrated on meiosis whilst the present work has been almost entirely upon somatic cells, the two sets of data supplement each other. In any case, in a field where

<sup>1</sup> E.g. aneuploidy in related individuals of *Matthiola incana*, Frost and Mann (11), and of *Zea mays*, Randolph (38); polyploidy similarly by many authors, as in *Nasturtium* in the present work.

adequacy and accuracy of information are so essential, but so difficult to attain (to judge by the frequent disagreement of cytological records) an independent testimony adds greatly to the reliability of results. No apology is, therefore, given for the presentation of the combined data.

#### MATERIALS AND METHODS.

Most of the plants have been from botanic gardens, being either fixed *in situ* or obtained as seeds which were grown to maturity in Cambridge Botanic Garden. The source of each is indicated by a reference number, but, since greater interest often attaches to species of known origin, all those (some 40 odd) of which wild seed or plants were available have been further marked out by an asterisk. The difficulty of possible hybridity which is always present in garden material has to some extent been overcome by the simultaneous investigation of the same species from different sources. Some 40 odd species have been verified in this way.

Every care has been taken with identification. Most of the specimens have been compared with those in herbaria and with descriptions. Difficult cases have been referred to Kew or the British Museum. As a further precaution against any errors that may still remain, wherever possible an adequate herbarium specimen of the actual plant used has been kept, reference to which may be had on application to the Keeper of the Herbarium, The University, Manchester.

*Fixation.* Chromosome counts have in every case been made from root-tips, though some few have been supplemented from smear preparations of developing pollen. The fixative has always been some form of the now familiar chrom-acetic-formalin of Karpechenko and Langlet. The formula used in the University of Stockholm comprised equal parts of two solutions:—Solution A: 1 gram.  $\text{CrO}_3$ ; 65 c.c. water; 10 c.c. glacial acetic acid. Solution B: 40 c.c. commercial formalin; 35 c.c. water.

The value of this reagent lies not only in the quality of fixation and the way in which it can be adapted to individual requirements, but in the preservative action of the formalin, which obviates the necessity of immediate washing, which in the field may be impossible.

Two modifications have frequently been used profitably:—(1) With English reagents a slightly weaker solution of chromic acid seems preferable, and 65 c.c. of a solution of 1 gram. to 80 c.c. of water has been adopted, British Drug Houses' chemicals being those employed. (2) A generally weaker solution is obtained by halving the amount of both acetic acid and formalin in the above formula, and has often been of value where the strong solution has failed.

Wherever possible the tubes have been kept cool in water during fixation, since a low temperature appears to improve the result.

*Staining.* For the first 100 species investigated Heidenhain's iron-alum-haematoxylin was used, the most successful timing being :

- 4 hours mordant.
- $\frac{1}{2}$  hour washing in running water.
- Rinse in distilled water.
- 2-4 hours stain.
- Differentiate and dehydrate in the usual way.

In the later part of the work a modification of Newton's gentian violet has been used, which avoids the difficulty of a darkened cytoplasm which is frequently found in using haematoxylin on a formalin fixative. The timing adopted was the following :

- $\frac{1}{2}$  hour mordant in the alcoholic iodine (1 grm. KI + 1 grm. I in 100 c.c. 80 per cent. alcohol).
- Rinse in water.
- 10 minutes stain (saturated B.D.H. gentian violet).
- $\frac{1}{2}$  minute alcoholic iodine.
- Dehydrate and differentiate in clove oil in the usual way.

This modification allows greater control of the final colour, and permits a residue to remain in the cytoplasm when desired, without which the invisibility of small sections to the naked eye may be inconvenient. The gentian violet method appears to have the disadvantage, however, that preparations do not improve with age as do those stained with haematoxylin.

In all cases the best differentiation was effected by examining the preparations under a 'Pointolite' lamp dimmed with a neutral screen and limited to a contrasting wave length by a combination of yellow and green photographic colour screens.

Drawings were made by the aid of  $\frac{1}{12}$  in. oil-immersion objective N.A. 1.3 and a Zeiss camera-lucida with compensating ocular 18, giving a magnification of 2,800.

The work was carried out between October, 1927, and June, 1930, and was begun in the Botanical Institute of the Högskola in Stockholm, and continued in the Botany School, Cambridge, the Jodrell Laboratory, Kew, and the University of Manchester. Thanks are due to the authorities of all these laboratories, especially to Professor Rosenberg, Mr. Brooks, and Professor Weiss for much kindness and help, and to Professor Tischler for his generosity in giving the use of his private library and in publishing the interim report in his revised list of chromosome numbers (54).

Grateful acknowledgment must also be made to the authorities of those gardens where personal fixation of material was carried on, and also to all those donors of seed, both public and private, named or implied on p. 44. To the staff in the herbaria at Kew and the British Museum a debt is due for help in the identification of specimens. Finally especial thanks must be rendered to the staff of Cambridge Botanic Garden and to Miss Saunders and Miss Harding, of Cambridge, for assistance in the culture and harvesting of the plants.



## COMMENTS ON CYTOLOGICAL AND TAXONOMIC DETAILS.

('f' denotes 'fundamental number', see p. 537. The actual chromosome numbers will be found on p. 541 et seq.)

Tribus:<sup>1</sup> *ARABIDEAE*.

Subtribus: *Sisymbriinae*.

SISYMBRIUM Figs. 14-18.

DESCURAINIA f = 7. Figs. 19-20.

The two genera are cytologically indistinguishable. The chromosomes are small and usually show one pair at least with a marked subterminal constriction which may give a misleading appearance of additional chromosomes after the ordinary full-strength fixative. All such difficulties in both genera were clearly resolved by the use of the half-strength fixative. It is therefore suggested that both the records of Laibach (28) for *S. strictissimum* and of Jaretsky (22) for *S. supinum* are erroneous. In the former case the discrepancy suggests an error in identification of the plant, though it is not impossible that the Kew specimen may represent a local tetraploid race.

The distinctness of *Sisymbrella* as advocated by Schulz (46) is corroborated since the fundamental number is there undoubtedly 8.

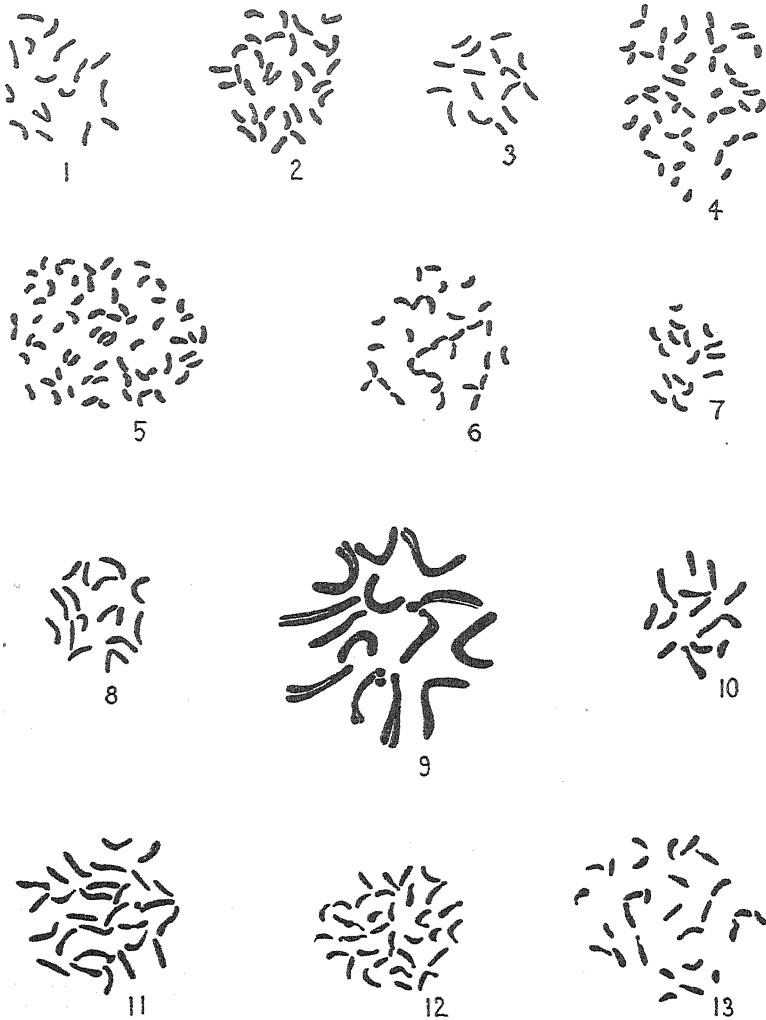
Subtribus: *Erysiminae*.

ERYSIMUM f = 8 and 7?. Figs. 27-9.

This genus is the most refractory to work with of any so far attempted. Fixation is uniformly difficult, though the half-strength fixative used very cold is the most generally successful. A greater difficulty is that of identification. The nomenclature of the forms in cultivation in botanic gardens is in a chaotic state, and it is very probable that hybridization is rife. Very little wild material has been available. It is therefore premature to stress taxonomic applications, though it seems probable that the cytology may become diagnostic in delimiting *Erysimum* from *Cheiranthus*. The two genera require similar technical treatment and both tend to show considerable diversity of chromosome size within the same cell, a fact which increases the difficulties of observation. The idea of a close affinity between them is therefore strengthened, but while the fundamental number in *Cheiranthus* is undoubtedly 7 that of *Erysimum* is, in part at least, 8. Should further investigation show that this distinction is generally maintained it would seem advisable to decide the disputed position of a species such as *E. linifolium* ( $2n = 14$ ) by transferring it definitely to the other genus.

The marked polyploidy between the forms studied is a noticeable feature, though it is possible that this is partly the result of cultivation and therefore of less real significance.

<sup>1</sup> The systematic arrangement is that of von Hayek (15) except where otherwise stated.



FIGS. 1-13. Tribus ARABIDEAE (excluding genera in 'Sisymbrieae' sensu O. E. Schulz). 1. *Barbarea intermedia*,  $2n = 16$ . 2. *Roripa sylvestris*,  $2n = 32$ . 3. *Roripa pyrenaica*,  $2n = 16$ . 4. *Nasturtium officinale*,  $2n = 48$ . 5. *Cardamine chenopodiifolia*,  $2n = 64$ . 6. *Cardamine flexuosa*,  $2n = 32$ . 7. *Cardamine amara*,  $2n = 16$ . 8. *Turritis glabra*,  $2n = 16$ . 9. *Bunias orientalis*,  $2n = 14$ . 10. *Tauscheria lasiocarpa*,  $2n = 14$ . 11. *Isatis tinctoria*,  $2n = 28$ . 12. *Armoracia rusticana*,  $2n = 32$ . 13. *Goldbachia laevigata*, var. *ascendens*,  $2n = 28$ .

Subtribus: *Cardamininae*.

BARBAREA  $f = 8$ . Fig. 1.

All the species examined are cytologically indistinguishable. The half-strength fixative is required. (Cf. *Sisymbrium*.)

RORIPA  $f = 8$ . Figs. 2 and 3.

See *Nasturtium* and *Armoracia*.

ARMORACIA  $f = 8$ . Fig. 12.

The cytology corroborates the desirability of separating this genus from *Cochlearia* and is in accordance with an affinity with *Roripa*, as suggested by Hegi (16) and von Hayek (15).

NASTURTIUM  $f = 8$ . Fig. 4.

The discovery of three polyploid forms (with somatic chromosome numbers of 32, 48, and 64) within the same taxonomic species is being made the subject of a special study and will not be further discussed here. Only the form with 48 somatic chromosomes is figured.

CARDAMINE  $f = 8$ . Figs. 5-7.

Representatives of eight out of the eleven sections in Schulz's monograph (43) have been examined and polyploidy is uniformly distributed. The tetraploidy of *C. flexuosa* with respect to the diploid *C. hirsuta* adds an interesting new diagnostic character for their separation. The octoploidy ( $2n = 64$ ) of both the sterile abnormal forms of *C. pratensis* in comparison with the tetraploidy of the normal form ( $2n = 32$ ) is also of interest.

Concerning the hybrid origin of some species, suggested by various authors (see Schwarzenbach (49), Leopold (30)), only the case of *C. Savensis* ( $2n =$  at least 80) has been freshly touched upon. Unfortunately, in addition to the alleged hybrid, only one of the supposed parents has been obtainable, *C. amara* ( $2n = 16$ ). The discrepancy in numbers gives some colour to Leopold's view that the two species cannot be directly connected, but in the absence of the other supposed parent, *C. enneaphylla*, it is impossible to be conclusive. On the other hand, the desirability of checking the morphological treatment of such a subject by including both cytological and genetical evidence is clearly brought out.

CARDAMINOPSIS  $f = 8$ . Jaretsky (21).

ARABIDOPSIS  $f = ?$ . Fig. 21.

The genus requires and deserves further study. The results of other investigators for *A. thaliana* ( $n = 5$ ) are exceedingly anomalous and, in spite of the number of records, should be confirmed by a somatic count. The diversity of the only other two species examined (see p. 543) prevents any general conclusions for the genus as a whole.

TURRITIS  $f = 8$ . Fig. 8.

*T. glabra*, the only species studied, is apparently in cultivation as two polyploid races, since Jaretsky (21) records 16 as the haploid number, while the plant available to the author had 16 as the diploid number.

Subtribus: *Arabidinae*.ARABIS  $f = 8$ . Jaretsky (21).AUBRIETIA  $f = 8$ . Jaretsky (21).Subtribus: *Isatidinae*.MYAGRUM  $f = 7$ . Jaretsky (22).ISATIS  $f = 7$ . Fig. 11.

All the forms examined are cytologically indistinguishable. They require the half-strength fixative or the presence of two subterminal constrictions, and a tendency for the chromosomes to elongate during fixation gives trouble. All except one were from botanic gardens, and the question of identification is very difficult since the genus has not recently been revised. The names given are as determined from Hegi (16) and by reference to the herbaria at Kew and the British Museum, but the original names are also appended. There seems no adequate reason why all should not be included as *I. tinctoria*. It would be of interest to be able to compare the idiogram of some other true species such as *I. alpina* All. from wild material.

TAUSCHERIA  $f = 7$ . Fig. 10.

The chromosome number is quite in accord with a systematic position of this genus near *Myagrum* and *Isatis*.

Subtribus: *Buniadinae*.BUNIAS  $f = 7$ . Jaretsky (21), Heitz (19), Håkansson (14). Fig. 9.

That the appearance of 'Sammelchromosomen' for *B. orientalis*, reported by Jaretsky, was due to a local occurrence of vegetative multiplication of chromosomes has already been shown by the other two authors. The present figure is included here for completeness and amply confirms their result.

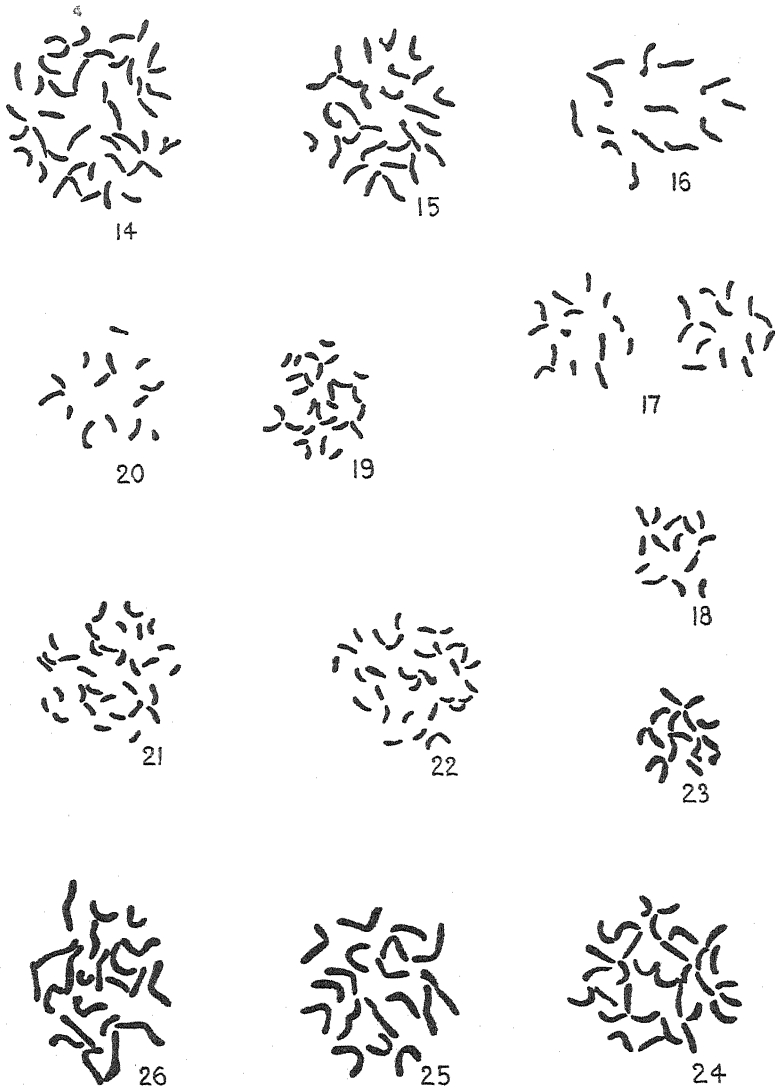
There is not sufficient knowledge of neighbouring genera to make the cytological evidence conclusive for taxonomy. An affinity between *Bunias* and *Goldbachia* is quite in harmony, but equally so is a grouping of *Bunias* with *Chorispora* (cf. Hegi (16)), placed by von Hayek in the *Brayinae* cf. diagrams on pp. 533 and 534. How important the difference in chromosome size may be as a criterion for separating *Bunias* from either genus is not known. There is, however, nothing to support Bonnier's hypothesis (4) of an affinity with *Coronopus* and *Cakile*.

GOLDBACHIA  $f = 7$ . Fig. 13.

*Genera added to the Sisymbriaceae by Schulz* (46). (See p. 535).

ONURIS  $f = 9$ . Fig. 25.BRAYA  $f = 8$ . Fig. 24.

The position here is greatly preferable to the one given by von Hayek (see Table II and p. 535), but it raises the question, which cannot yet be



FIGS. 14-26. 'Sisymbrieae' sensu O. E. Schulz and *Sisymbrella*. 14. *Sisymbrium runcinatum*,  $2n. = 42$ . 15. *Sisymbrium strictissimum*,  $2n. = 28$ . 16. *Sisymbrium irio*,  $2n. = 14$ . 17. *Sisymbrium sinapistrum*,  $2n. = 14$  (superposed anaphase plates). 18. *Sisymbrium Assoanum*,  $2n. = 14$ . 19. *Descurainia Menziesii*,  $2n. = 28$ . 20. *Descurainia Cumingiana*,  $2n. = 14$ . 21. *Arabidopsis pumila*,  $2n. = 32$ . 22. *Sisymbrella dentata*,  $2n. = 32$ . 23. *Sisymbrella aspera*,  $2n. = 16$ . 24. *Braya alpina*,  $2n. = 32$ . 25. *Onuris graminifolia*,  $2n. = 18$ . 26. *Xerodraba pycnophylloides*,  $2n. = 22$ .

answered, as to whether  $f = 8$  as here, or  $f = 7$  as in *Sisymbrium* itself, is the more primitive for the group.

XERODRABA  $f = 11$ . Fig. 26.

*Genera separated from von Hayek's genera by Schulz (46).*

HUGUENINIA  $f = 7$  or  $8$  (?).

Uncertainty is due to difficulty of fixation.

SISYMBRELLA  $f = 8$ . Figs. 22, 23.

The separation of this genus from *Sisymbrium* ( $f = 7$ ) is clearly supported.

Tribus: *ALYSSEAE*.

Subtribus: *Hesperidinae*.

CHEIRANTHUS  $f = 7$ . Fig. 30.

A difficult genus both for fixation, observation and identification. The cultivated species have proved unworkable, but the fundamental number for the genus seems to be firmly established from wild material. See *Erysimum* p. 513.

HESPERIS  $f = 7$  and  $6$ . Figs. 31-4.

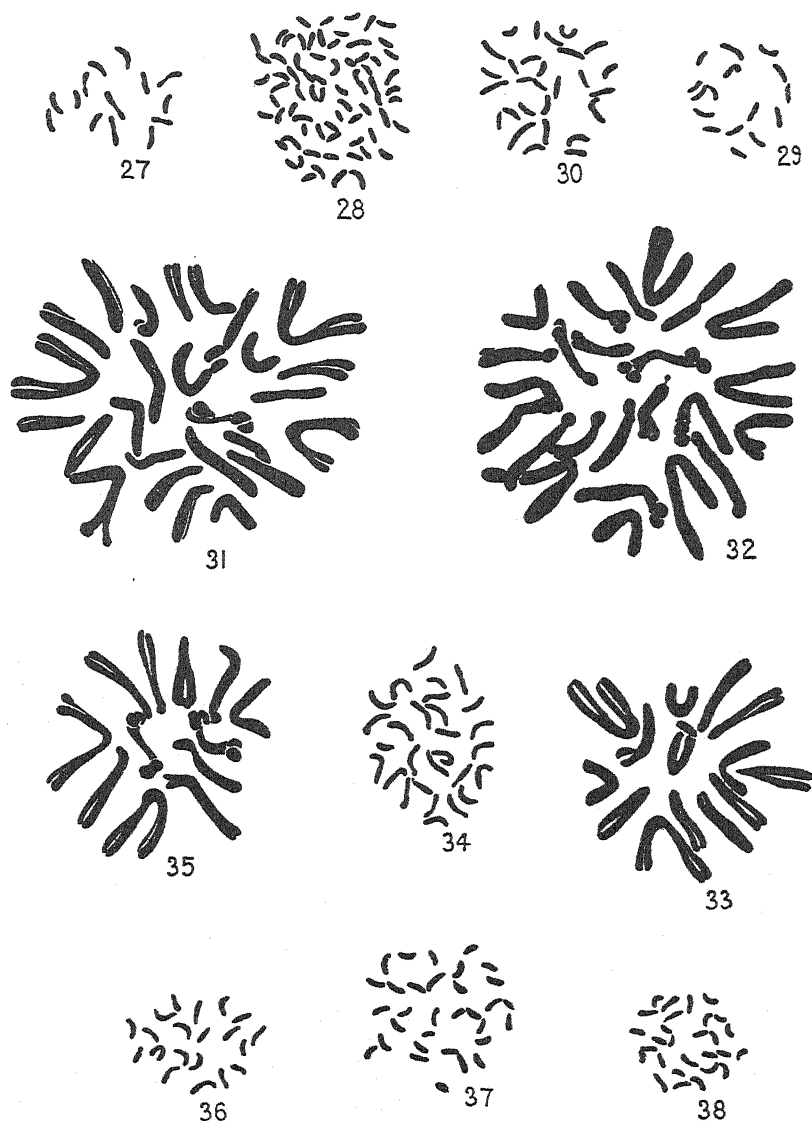
The genus is outstanding among the Crucifers for the large size and varied form of the chromosomes. No attempt has been made here towards a comparative survey of the shapes of individual chromosomes, though it is hoped later to do so. The great disparity in size between the chromosomes of *H. lutea* Max. and the others is noteworthy.

The record of Jaretsky (21) of a haploid number of 14 for *H. matronalis* is almost certainly an error and due to the presence of a pair of very large chromosomes with median constrictions. That these are two and not four has been clearly determined here in plants from four independent sources and in the homotype division in smear preparations of pollen from a fifth. The plant at present in cultivation would seem to be a tetraploid on 6 and to have been derived originally by aneuploid loss of a chromosome from a form with the (still predominant) haploid number 7. This is an interesting parallel with what has previously been demonstrated in *Matthiola*, and a comparison with Fig. 35 further suggests the close affinity with that genus.

Affinity with *Erysimum* and *Malcolmia* is by no means so apparent. (See Figs. 27-38).

MALCOLMIA  $f = 7$  and aneuploid. Figs. 36-8.

The general appearance of the chromosomes is strikingly unlike that in *Hesperis*. The irregular numbers, though suggestive of the aneuploid processes shown in both *Hesperis* and *Matthiola*, should here be interpreted rather as an indication of the need for systematic revision of the genus (as noted by von Hayek). The occurrence of aneuploidy here cannot be regarded as established until this is done.



FIGS. 27-38. Tribus ALYSSEAE; subtribus *Hesperidinae*. 27. *Erysimum linifolium*,  $2n = 14$ . 28. *Erysimum canescens*,  $2n = 72$ . 29. *Erysimum cheiranthoides*,  $2n = 16$ . 30. *Cheiranthus cinereus*,  $2n = 28$ . 31. *Hesperis sylvestris*,  $2n = 26$ . 32. *Hesperis matronalis*,  $2n = 24$ . 33. *Hesperis tristis*,  $2n = 14$ . 34. *Hesperis lutea*, Max.  $2n = 28$ . 35. *Matthiola odoratissima*,  $2n = 12$ . 36. *Malcolmia littorea*,  $2n = 20$ . 37. *Malcolmia chia*,  $2n = 32$ . 38. *Malcolmia africana*,  $2n = 28$ .

MATTHIOLA  $f = 7$  and 6. Fig. 35. Jaretsky (22), Manton (34).

The aneuploid evolutionary progress within the genus from a fundamental number of 7 to one of 6 has been reported and discussed elsewhere,

and there is nothing here to add to the list which is quoted in the summary. Attention may be drawn to the strong resemblance, both in chromosome appearance and evolutionary behaviour, between *Matthiola* and *Hesperis*. To make this clear, one of the three figures already published has been reproduced (Fig. 35).

Subtribus: *Brayinae*.

BRAYA  $f = 8$ . See p. 516.

CHORISPORA  $f = 7$ . Fig. 49.

Subtribus: *Euclidiinae*.

EUCLIDIUM  $f = 7$ . Fig. 52.

Subtribus: *Lunariinae*.

RICOTIA  $f = 7?$ . Fig. 39.

See *Lunaria*.

LUNARIA  $2n = 30?$  Figs. 40, 41.

The earlier work of Laibach (28) that gave a number of 24 to *Lunaria annua* is undoubtedly an error. The only uncertainty is due to the fact that in *L. rediviva* two of the chromosomes are very small and round and may possibly correspond to the extra bodies of *Iberis garrexiana* and *saxatilis*. This is not so evident in *L. annua*, but the material has not been sufficiently abundant for certainty.

In other respects the resemblance to *Ricotia* is close. The difference in chromosome size, as well as number, is very marked between the *Lunariinae* and the *Alyssinae* (Diagram II, p. 534).

Subtribus: *Alyssinae*.

FIBIGIA  $f = 8$ . Fig. 42.

All the species commonly grown in gardens under the name of *Farsetia* belong to this genus in the sense used by von Hayek. The two species investigated agree with the rest of the *Alyssinae*.

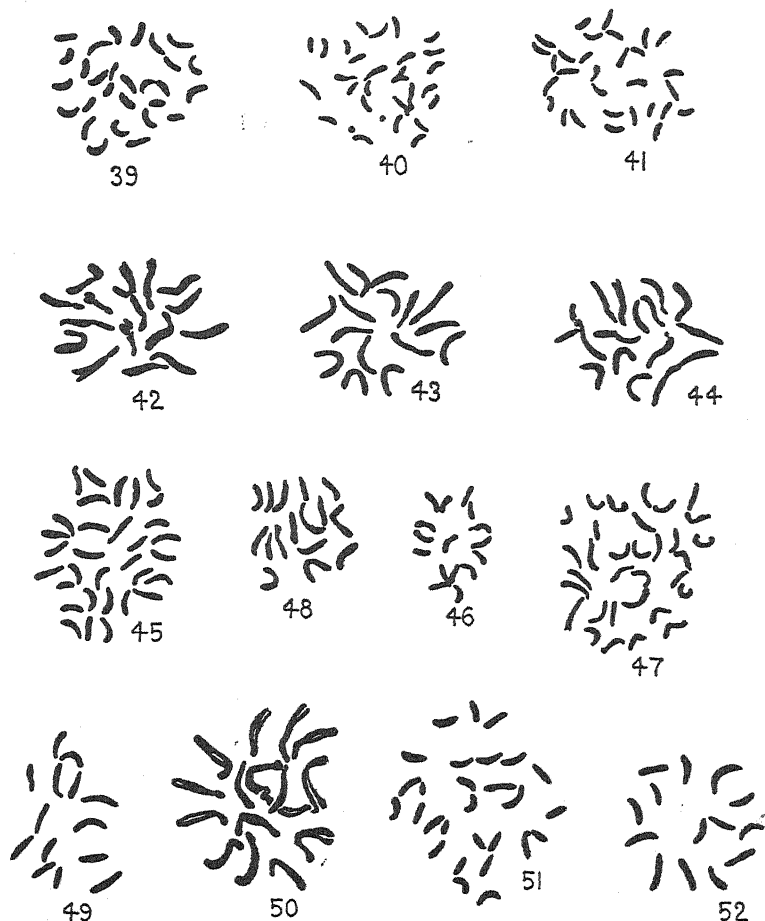
BERTEROA  $f = 8$ . Fig. 43.

The chromosomes of the two species examined are slightly larger than is the general rule in *Alyssum*. In this way they seem to be more distinct than any of the other genera whose amalgamation with that genus has been debated. It is doubtful, however, how much weight is to be attached to differences of size.



ALYSSUM  $f = 8$ . Figs. 44-7.

Confusion of specific nomenclature made it inconvenient to follow von Hayek's grouping in detail, and species variously attributed to his genera



FIGS. 39-52. Tribus ALYSSEAE (*continued*). *Hesperidinae* and *Braya* excluded. 39. *Ricottia lunaria*,  $2n = 28$ . 40. *Lunaria rediviva*,  $2n = 30$ . 41. *Lunaria annua*,  $2n = 30$ . 42. *Fibigia clypeata*,  $2n = 16$ . 43. *Berteroa Incana*,  $2n = 16$ . 44. *Alyssum montanum*,  $2n = 16$ . 45. *Alyssum pyrenaicum*,  $2n = 32$ . 46. *Alyssum Borzaceanum*,  $2n = 16$ . 47. *Alyssum spinosum*,  $2n = 32$ . 48. *Schievereckia podolica*,  $2n = 16$ . 49. *Chorispora tenella*,  $2n = 14$ . 50. *Vesicaria utriculata*,  $2n = 16$ . 51. *Thysanocarpus curvipes*,  $2n = 28$ . 52. *Euclidium tataricum*,  $2n = 14$ .

*Ptilotrichum* and *Koniga* are here all included as *Alyssum*. The chromosomes are on the whole rather uniform, and it is doubtful if taxonomic difficulties can here be solved by their study.

VESICARIA  $f = 8$ . Fig. 50.

The position near *Alyssum* is satisfactory and emphasizes the

vegetative polyploidy in one root of *C. pinnatifida* (Figs. 77 and 78) and the general prevalence of vegetative propagation<sup>1</sup> by the rhizome in gardens suggests that the cytological condition of the wild species may in some instances have been artificially obscured. But in any case the great discrepancy between the apparently sexually sterile *C. orientalis* ( $2n = 120$ ) of gardens and the form *C. koktebelica* ( $2n = 30$ ), which Schulz (44) regards as merely a variety of the former, is of interest. Any wild material would be welcomed.

CALEPINA  $f = 7$ . Fig. 69.

The great diversity in the two records for *C. irregularis* ( $n = 7$  here, but  $= 21$  Jaretsky (22)) may be due to a tendency to intraspecific polyploidy as in *Crambe*. The species should, however, be reinvestigated from wild material.

RAPHANUS  $f = 9$ . Fig. 63.

COSSONIA (*Raffenaldia*)  $f = 7$ . Fig. 62.

Von Hayek's derivation of *Cossonia* from *Raphanus* is not supported, both the number and form of the chromosomes showing much more resemblance to *Morisia* in accordance with the older view.

#### Subtribus: *Vellinae*.

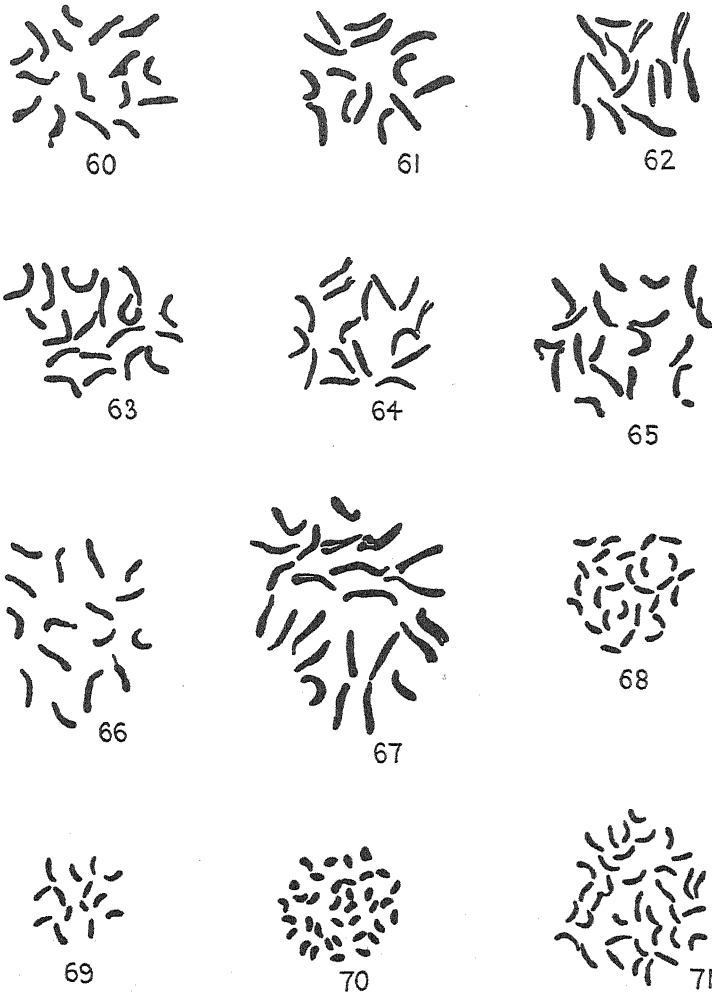
CARRICHTERA  $f = 8$ . Fig. 66.

Since both *C. 'vella'* and *C. 'annua'* are really the same species to which Schulz (45) gives the name of *C. annua*, little significance is attached to the discrepancy between some of the present results and one of Jaretsky's (21) (garden forms of *C. annua* being respectively reported as  $2n = 32$  and  $2n = 16$ ). Polyploid cultural forms are probably involved. The two different plants in the present instance were apparently identical, and there was no obvious trace of a gigas effect. Unfortunately, the tetraploid plant died before a herbarium specimen was made, and an accurate comparison was therefore impossible.

SUCCOWIA  $2n = 36$ . Figs. 70, 71.

The fact that the same result was obtained for vegetative counts of plants from two different sources suggests that Jaretsky's result (22) ( $n = 16$ ) may be an error. The species appears to be a tetraploid on 9. The strikingly different appearance of the chromosomes in the two figures is apparently due to the physiological state of the cell, Fig. 70 being from a very young radicle richly stocked with fat.

<sup>1</sup> The garden species seems to be sexually sterile. Seed from every available botanic garden has been attempted, but in no case has germination been obtained.



FIGS. 60-71. Tribus: BRASSICEAE (continued). (*Brassicinae* and *Crambe* excluded). 60. *Reboudia erucarioides*, 2 n. = 16. 61. *Morisia hypogaea*, 2 n. = 14. 62. *Cossonia africana*, 2 n. = 14. 63. *Raphanus landra*, 2 n. = 18. 64. *Rapistrum rugosum*, 2 n. = 16. 65. *Cakile maritima*, 2 n. = 18. 66. *Carrichtera vella*, 2 n. = 16. 67. *Orychophragmus violaceus*, 2 n. = 24. 68. *Moricandia arvensis*, 2 n. = 28. 69. *Calepina irregularis*, 2 n. = 14. 70. *Succowia balearica* (radicle), 2 n. = 36. 71. *Succowia balearica* (adult root), 2 n. = 36.

Subtribus: *Moricandiinae*.

CONRINGIA f = 7. Jaretsky (21).

MORICANDIA f = 7. Fig. 68.

ORYCHOPHRAGMUS f = 6. Fig. 67.

This is another good instance of aneuploid loss in a derived genus (cf. *Thysanocarpus*, &c.).

Tribus: *LEPIDIEAE*.Subtribus: *Lepidiinae*.*LEPIDIUM*  $f = 8$ . Figs. 95-7.

A very uniform polyploid genus.

*HYMENOPHYSA*  $f = 6$ . Fig. 98.*CORONOPUS*  $f = 8$ . Fig. 99.*BISCUTELLA*  $f = 8$  and 9. Figs. 100, 101.

*B. laevigata*, with both 18 and 36 chromosomes as opposed to the majority of species in which the number is 16, presents an interesting evolutionary problem which is being further studied. It is the clearest instance yet found in the family of an aneuploid gain in a derived type. (Contrast with *Hesperis* p. 10, *Thysanocarpus* p. 14, &c.)

Subtribus: *Iberidinae*.*HUTCHINSIA*  $f = 6$ . Figs. 83-5.

The chromosomes are very uniform in number and size. They are among the smallest in the family, but show considerable resemblance to those of *Aethionema*.

The inclusion of *H. procumbens* in the genus is undoubtedly correct, the chromosome number being incompatible with its assignment to *Capsella*. That the count is accurate is made additionally certain by the occurrence of local vegetative polyploidy in one root in which both 12 and 24 chromosomes could clearly be made out (Fig. 85).

*IBERIS*  $f = 7$  and 11 (and 8?). Figs. 86-94.

The chromosomes of all the species with multiples of 11 are smaller and more uniform in shape than those with 14. In the latter they are sufficiently large and varied for individual differences to be made out.

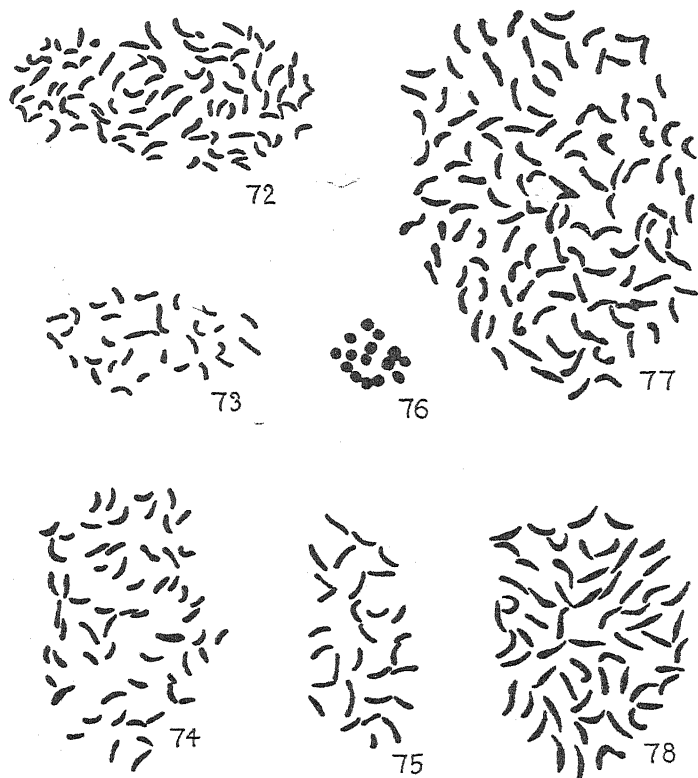
The occurrence of two such distinct chromosome types (see p. 549) suggests the need for systematic revision of the sections at any rate. Whether the third number, 8, is real or merely due to cultural races (as in *Matthiola incana*: Frost and Mann (11)) is not certain. Neither of the two previous reports, *I. pinnata* (Laibach (28)), and *I. amara* (Jaretsky),<sup>1</sup> in which 16 somatic chromosomes are given, have been confirmed.

The occurrence of one loose fragment has been noted in *I. semperflorens* var. *garrexiana* from Kew, and two in *I. saxatilis* from Copenhagen. The fragments are in addition to the normal full complement of chromosomes and are distinguished by small size and spherical shape. Whether

<sup>1</sup> See Tischler (54).

they are constant features in nature or only individual peculiarities of the plants used is not known.

If *I. corifolia* is really a variety of *I. saxatilis*, as proposed by Hegi (16), its peculiar number (apparently 50) may possibly be attributed to frag-



FIGS. 72-8. *Crambe*. 72. *Crambe abyssinica*,  $2n = 90$ . 73. *Crambe koktebelica*,  $2n = 30$ . 74. *Crambe maritima*,  $2n = 60$ . 75. *Crambe fruticosa*,  $2n = 30$ . 76. *Crambe fruticosa* heterotype meiosis,  $n = 15$ . 77, 78. *Crambe pinnatifida*, same root,  $2n = 120$  and  $60$ .

mentation of some chromosomes in a tetraploid ( $2n = 44$ ). The irregularity of chromosome size gives colour to this idea, but both species should be reinvestigated and meiosis as well as mitosis be studied.

The cytology gives no clear indication of the external affinities of the genus.

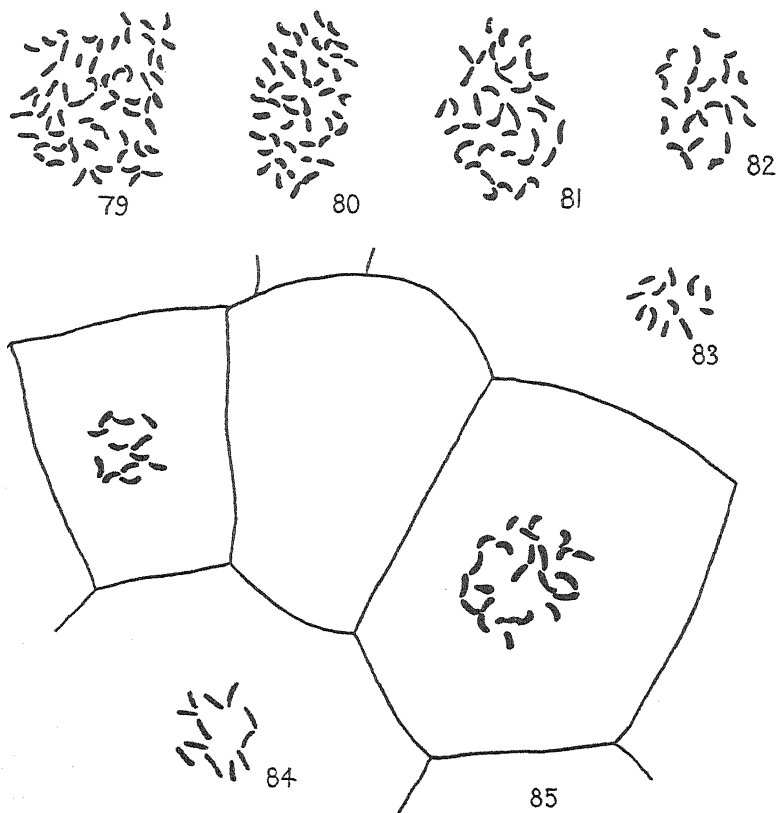
AETHIONEMA  $f = 6$ . Figs. 79-82.

This genus is very uniformly polyploid, and the series fairly complete. The fundamental number is shared with no other genus except *Hutchinsia*. The distinctness of *Aethionema* from *Eunomia* is strongly corroborated.

Subtribus: *Thlaspidinae*.

COCHLEARIA  $f = 7$ . Crane and Gairdner (8). Fig. 102.

The one additional species (garden material of *C. glastifolia*,  $2n = 38$ ) is aberrant, as are the previous reports (loc. cit.) for *C. anglica* and *C. micacea*, all suggesting hybridity.



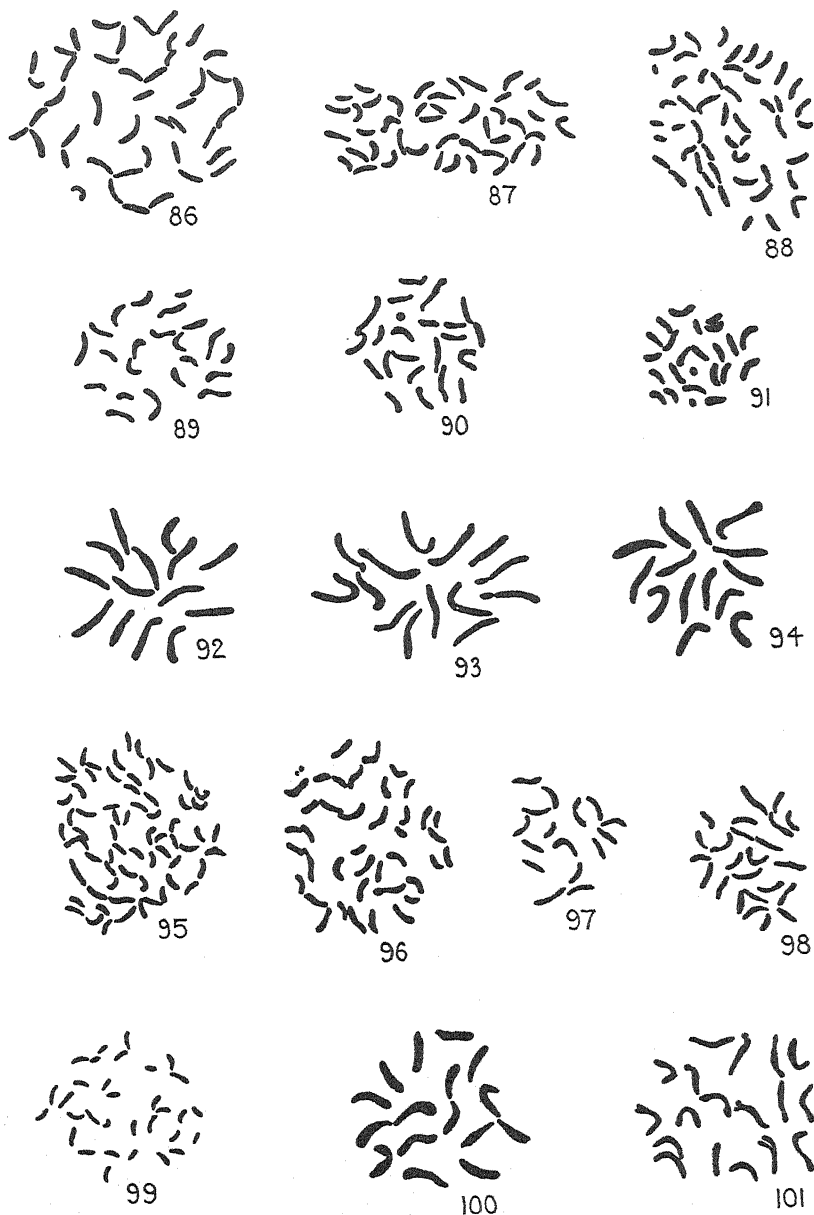
FIGS. 79-85. *Aethionema* and *Hutchinsia*. 79. *Aethionema cordatum*,  $2n = 60$ . 80. *Aethionema grandiflorum*,  $2n = 48$ . 81. *Aethionema Thomasianum*,  $2n = 36$ . 82. *Aethionema schistosum*,  $2n = 24$ . 83. *Hutchinsia petraea*,  $2n = 12$ . 84. *Hutchinsia alpina*,  $2n = 12$ . 85. *Hutchinsia procumbens*,  $2n = 12$  and  $24$ .

The distinctness of *Armoracia rusticana* (= *Cochlearia Armoracia*) is clearly corroborated (cf. p. 515).

COLUTEOCARPUS  $f = 7$ . Fig. 103.

The cytology corroborates von Hayek's placing of this genus near *Cochlearia*, rather than near *Alyssum* as on the older view.

IONOPSISIDIUM  $f = 8?$  ( $2n = 24$  and  $32$ , Chiarugi (6)).



FIGS. 86-101. Tribus LEPIDIEAE; subtribus *Lepidiinae* and *Iberis*. 86. *Iberis correaefolia*,  $2n. = 44$ . 87. *Iberis garrexiana*,  $2n. = 44$ . 88. *Iberis corifolia* ( $2n. =$  modified 44?). 89. *Iberis pruiti*,  $2n. = 22$ . 90. *Iberis semperflorens*, var. *garrexiana*,  $2n. = 22 + 1$ . 91. *Iberis saxatilis*,  $2n. = 22 +$  two fragments. 92. *Iberis amara*,  $2n. = 14$ . 93. *Iberis taurica*,  $2n. = 14$ . 94. *Iberis odorata*,  $2n. = 14$ . 95. *Lepidium draba*,  $2n. = 64$ . 96. *Lepidium crassifolium*,  $2n. = 40$ . 97. *Iberis armoracium*,  $2n. = 16$ . 98. *Hymenophyllum pubescens*,  $2n. = 24$ . 99. *Coronopus procumbens*,  $2n. = 32$ . 100. *Biscutella erigerifolia*,  $2n. = 16$ . 101. *Biscutella glacialis*,  $2n. = 18$ .

EUNOMIA  $f = 7$ . Figs. 108, 109.

The distinctness of this genus from *Aethionema* is strongly corroborated, the chromosomes differing markedly in number, size, and shape (cf. p. 527).

THLASPI  $f = 7$ . Figs. 104, 105.

Here, as in *Alyssum*, the cytological characters are too uniform to be of much service in determining specific boundaries, except in the one case of delimiting *T. alpestre* ( $2n = 14$ ) from *T. montanum* ( $2n = 28$ ).

The chromosomes, as in *Sisymbrium*, frequently show a subterminal constriction on a single pair, and require the half-strength fixative.

TEESDALIA  $f = 9?$  ( $2n = 36$ ). Fig. 110.

This number does not fit very logically into the prevailing cytological plan of either the *Thlaspidinae* ( $f = 7$ ) or the alternative *Capsellinae* ( $f = 8$  and  $7$ ). It cannot be decided without experiment whether *Teesdalia nudicaulis* is a hexaploid on 6 or a tetraploid on 9. The cytological evidence is, therefore, inconclusive for the systematic position (see Diagram IV).

PELTARIA  $f = 7$ . Figs. 106, 107.

The cytological evidence does not favour the removal of this genus to the *Lunariinae*, as advocated by Calestani (5).

#### Subtribus: *Capsellinae*.

CAMELINA  $f = 8$ . Fig. 111.

Extreme confusion still prevails as to the specific division of the genus. Hegi (16) considers that all the forms studied here should be referred to *C. sativa* (L.) Cr. Other recent floras make various independent species. Tedin (52) from genetical evidence would select *C. microcarpa* as a true species.

Cytologically, all forms so far examined are indistinguishable. There seems little doubt that the chromosome number is 40, in spite of a report of 21 haploid chromosomes in *C. 'alyssum'* by Jaretsky (21). That author admits that only 20 were visible in the homotype division, and the appearance of 21 in a few heterotype figures must have been due to slight irregularity in the time of separation of the members of one pair.

The correction of Jaretsky's count makes unnecessary his assumption of an evolutionary sequence from *Capsella* to *Camelina*, which, from the structure of the fruit, seems highly improbable.

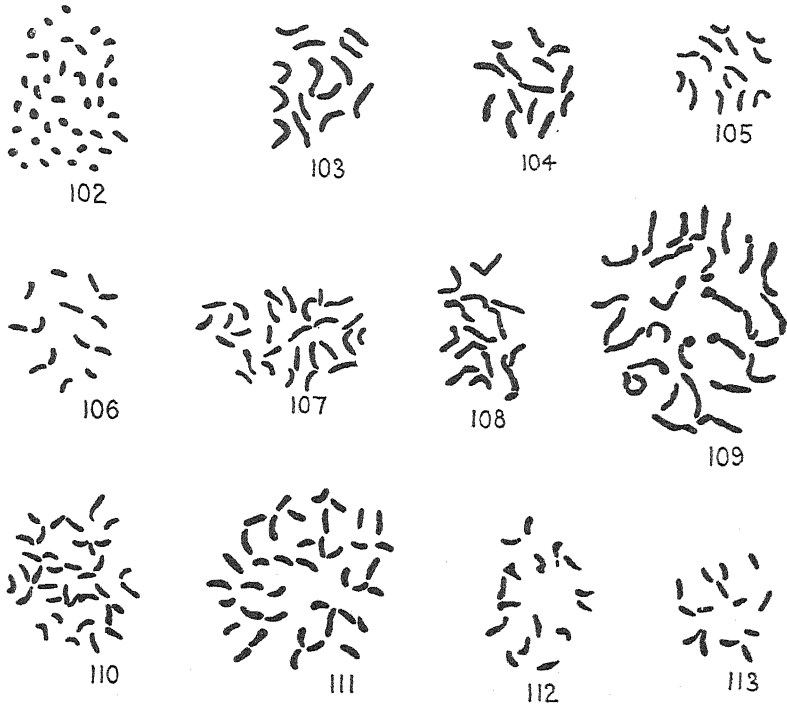
CAPSELLA  $f = 8$ . Fig. 112.

The count of *C. grandiflora* figured above was made before the work of Hill (20) and Shull (48) were available. Shull's view of the generic



distinctness of '*Capsella*' *procumbens* has been fully borne out, and Hegi's grouping of it with *Hutchinsia* (see p. 526) has been shown to be correct.

VOGELIA (= NESLIA)  $f = 7$ . Fig. 113.



FIGS. 102-113. Tribus LEPIDIEAE (*continued*). Subtribus *Thlaspidinae*. 102. *Cochlearia glastifolia*,  $2n = 38$ . 103. *Coluteocarpus reticulatus*,  $2n = 14$ . 104. *Thlaspi arvensis*,  $2n = 14$ . 105. *Thlaspi cepaeifolium*,  $2n = 14$ . 106. *Peltaria turkmena*,  $2n = 14$ . 107. *Peltaria alliacea*,  $2n = 28$ . 108. *Eunomia oppositifolia*,  $2n = 14$ . 109. *Eunomia iberidea*,  $2n = 28$ . 110. *Teesdalia nudicanlis*,  $2n = 36$ . 111. *Camelina linicola*,  $2n = 40$ . 112. *Capsella grandiflora*,  $2n = 16$ . 113. *Vogelia paniculata*,  $2n = 14$ .

### Tribus: SCHIZOPETALEAE.

#### Subtribus: *Physariinae*.

LESQUERELLA  $2 = 10, 18, 12, ca 50$ . Figs. 114-6.

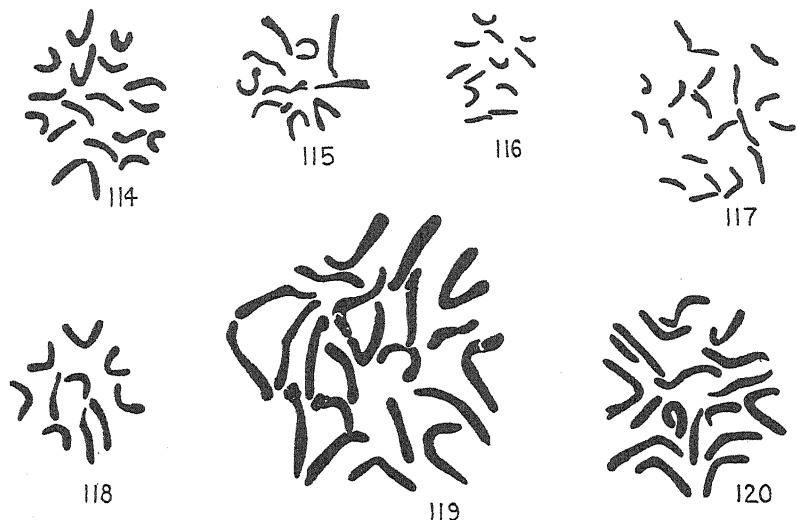
This genus would repay study by some one to whom the wild species were available. At present no general conclusions can be drawn except corroboration of the distinctness from the Old World genus *Vesicaria* with which it was formerly confused.

#### Subtribus: *Stenopetalinae*.

STENOPETALUM  $f = 5$ . Fig. 118.

#### Subtribus: *Schizopetalinae*.

SCHIZOPETALUM  $2n = 18$ . Fig. 120.

Tribus: *HELIOPHILEA*.*HELIOPHILA* f = 10. Fig. 117.Tribus: *CREMOLOBEAE*.*MENONVILLEA* f = 11 Fig. 119.

FIGS. 114-20. 114. *Lesquerella grandiflora*, 2 n. = 18. 115. *Lesquerella argentea*, 2 n. = 10. 116. *Lesquerella gracilis*, 2 n. = 12. 117. *Heliophila linearifolia*, 2 n. = 20. 118. *Stenopetalum lineare*, 2 n. = 10. 119. *Menonvillea Gayi*, 2 n. = 22. 120. *Schizopetalum Walkeri*, 2 n. = 18.

## PHYLOGENY IN THE CRUCIFERAE.

The problem of the origin of the Cruciferae has not been approached here, for no members of the group Thelypodieae which lies nearest to the Capparidaceae have been available, whilst the genera nearest to the Papaveraceae (*Subularia*, *Pringlea*, and *Arabidopsis*, according to Calestani (5)) are also either unknown or uncertain.

Within the family no attempt has been made to draw up a phyletic scheme on a basis of the chromosomes alone. For this far greater detail would be required, and in many parts of the family the cytological uniformity is so great that the attempt would be unprofitable. On the other hand, in the Brassicinae at least there is considerable likelihood that fuller cytological knowledge may indicate generic boundaries, and enable phylogeny to be traced with some degree of certainty.

Existing systematic opinions have, therefore, throughout been used as the basis of discussion, and the new data have supplied critical, rather than constructive, evidence. Detailed taxonomic problems have already been

considered categorically in the preceding pages, and the more important positive conclusions are specifically enumerated in the Summary. The

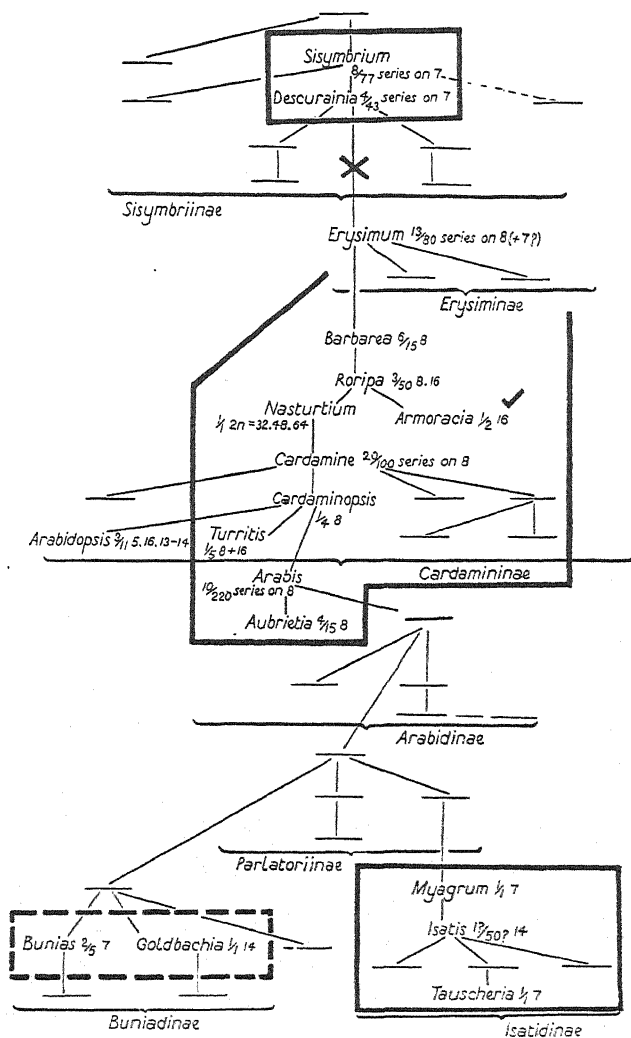


DIAGRAM I. Tribus : ARABIDEAE.

evidence bearing on general phylogeny, on the other hand, can most conveniently be presented diagrammatically.

*Explanation of diagrams.* Diagrams I-IV show the general cytological results superimposed upon the most recent complete phyletic scheme of the family, namely that of von Hayek (15). The nomenclature and the *thin* phylogenetic lines are his, though, where a genus is not known at all cytologically, its name is replaced by a thin horizontal line. This shows

at a glance the extent of the field which is still unexplored. In addition to the genera named, von Hayek's tribes Schizopetaleae (17 genera, of which 3 are represented), Pringleae (1 unknown genus), Heliophileae

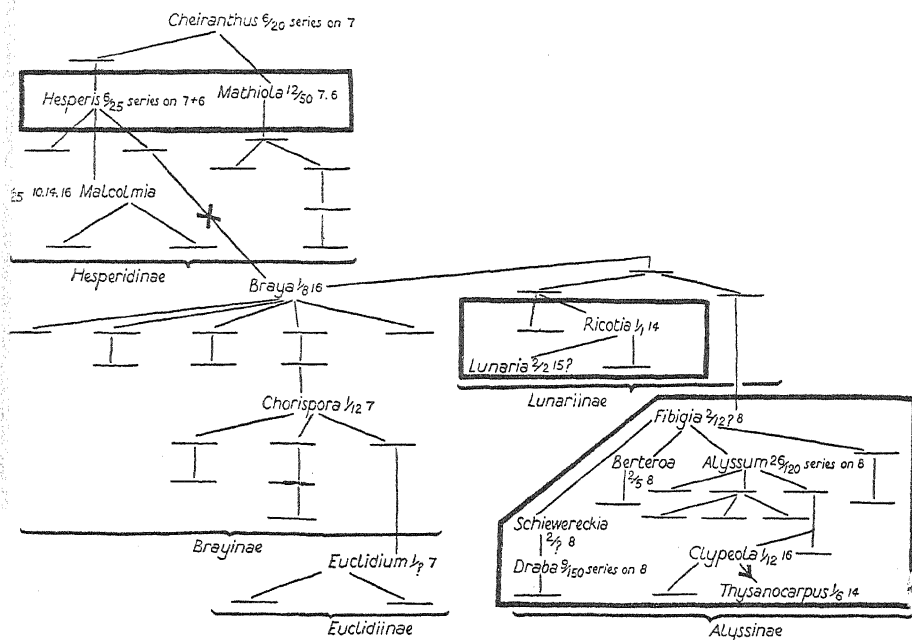


DIAGRAM II. Tribus: ALYSSEAE.

(6 genera, of which 1 is known), Cremolobeae (5 genera, of which 1 is represented), and Chamireae (1 unknown genus), constitute relatively untouched groups that have not been shown at all in the diagrams. On the other hand, new genera created since 1911 (e.g. *Sisymbrella*, etc.) have not been inserted.

The *thick type* represents the cytological views of affinity, and the following symbols have been used:

(i) A *continuous line* round a group of genera implies that they form a cytologically natural group.

(ii) A *discontinuous line* implies that a relationship is harmonious with the chromosomal evidence, but that this is so far inconclusive.

(iii) A *question mark* indicates that a given phyletic line cannot easily be reconciled with the cytology.

(iv) A *cross* through a phylogenetic line indicates where definite support is given to some alternative view (to be mentioned below).

(v) An *arrow-head* records an instance where a definite direction is assignable, on cytological grounds, to an evolutionary sequence, the point of the arrow being towards the more derived type in each case.

(vi) A *tick* against a genus denotes that its right to an independent status has been disputed, but that the cytology favours its retention.

The actual cytological facts are expressed numerically. The extent to which a genus is known is expressed as a fraction, of which the *numerator* denotes the number of species examined, while the *denominator* represents the total number of species contained. This statistical information has been drawn from Willis's Dictionary (57), except where the requisite volumes of Das Pflanzenreich have been available. Where extreme systematic confusion is known to exist the denominator is replaced by a question mark. The remaining *figures* denote the actual gametic numbers found, except where otherwise stated. In most cases these are obtained by calculation from the observed somatic numbers.

*Conclusions.* On the whole, the agreement on broad lines is striking. It is further interesting to note that many of the discrepancies between cytology and von Hayek's system are considerably lessened in the more recent, though unfortunately still incomplete, work of O. E. Schulz in Das Pflanzenreich. His principal emendations are:

(1) Von Hayek's Sisymbriinae are removed from his Arabideae, and made into a separate parallel tribe, the 'Sisymbrieae'. Diagram I shows that this avoids the assumption of a chromosomal evolution of fundamental numbers 7 to 8 to 7, which seems artificial. The new view is, therefore, preferable.

(2) The 'Sisymbrieae' are somewhat enlarged from the older Sisymbriinae, and include, among other old genera, *Arabidopsis* (see Diagram I) and *Braya* (see Diagram II). This position of *Braya* at the base of the 'Sisymbrieae' is much to be preferred. There seems little in common between the modern Hesperidinae and the modern Alyssinae (the Lunariinae are equally distinct) either in chromosome morphology or number, but *Braya* offers no point of contact.

There is no doubt, therefore, that the phyletic accuracy of the taxonomic system is gradually improving.

## EVOLUTIONARY SIGNIFICANCE OF CHROMOSOME CHANGES IN THE CRUCIFERAE.

No detailed evidence has been obtained concerning the bearing of changes in chromosome size or shape on the origin of new phenotypes. Suitable ground for such work is, however, probably to be found in *Matthiola*, *Hesperis*, and *Iberis*, the chromosomes in all being large enough for a study of individual morphology and interesting interspecific size-differences being already recorded in the last two.

With regard to changes in chromosome number the Crucifers have

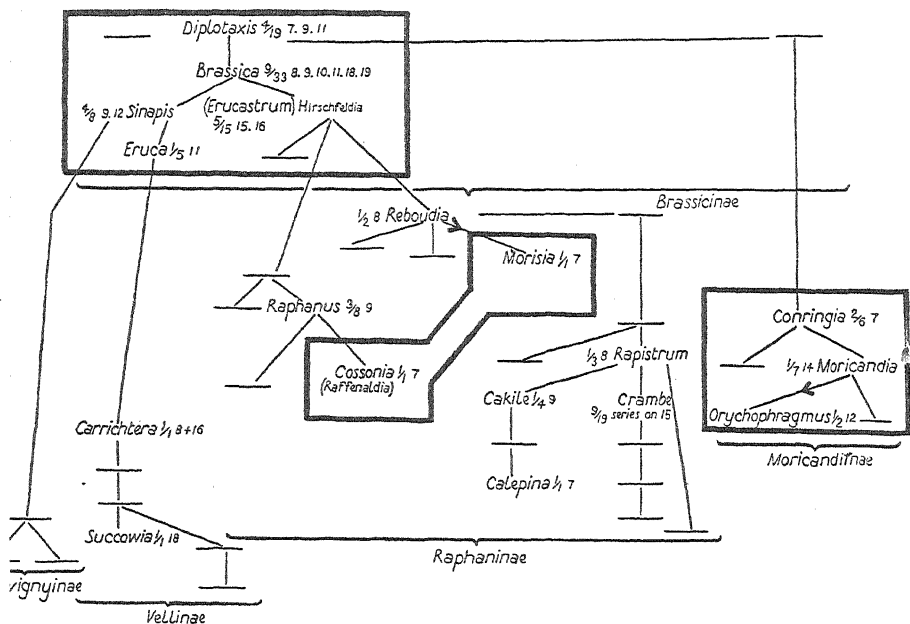


DIAGRAM III. Tribus: BRASSICEAE.

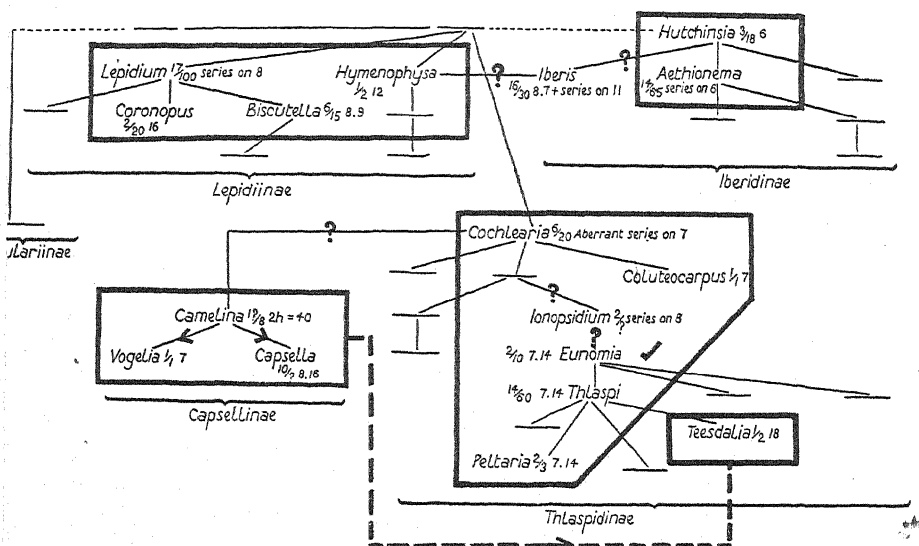


DIAGRAM IV. Tribus: LEPIDIEAE.

experienced cytological developments resulting in both aneuploidy and polyploidy.<sup>1</sup> The general survey has further brought out clearly some characteristic differences in the distribution of these conditions among the taxonomic units, which remain as facts even if the deductions concerning their evolutionary significance prove to be erroneous. These two cytological states will be briefly considered in turn.

*Aneuploidy*, as a relation between the species of the same genus, is not common, though, where it does occur (in the Brassicinae, and apparently also in the Hesperidinae), it is so well marked as to be the prevailing condition, polyploidy being rare if not absent. On the other hand, throughout the family aneuploidy is essentially the relation between the fundamental numbers of genera.

The fundamental number of a genus is that gametic number which there is reason to accept as the most primitive for the genus, and from which the actual numbers found can be derived. In an aneuploid group like the Brassicinae, where gametic numbers 7, 8, 9, 10, 11 occur, it is not possible to determine the fundamental from the chromosome numbers alone, since both gain and loss are possible and, further, an aneuploid change as such may be reversible. On the other hand, in a polyploid genus (comprising the majority of the Crucifers) the fundamental number very frequently coincides with the lowest gametic number found, the logical relationship to it is quite clearly seen in the bulk of the species composing the genus, and the same number is often shared by a whole group of neighbouring genera. Where the same number is not shared the relation is almost invariably aneuploid rather than polyploid.

On the whole, aneuploid loss, usually of one chromosome in the fundamental complement, is the more frequent, e.g. *Thysanocarpus*, *Vogelia*, *Isatidinae*, a fact which the experimental production of aneuploids would not lead one to expect. On the other hand, gain has been clearly demonstrated within the one genus *Biscutella*, and apparently in *Teesdalia* and *Succowia*.

The fact that, in the Cruciferae, *genera rather than species have, in their initiation, been involved in aneuploid changes*, seems to indicate that there may be a peculiar evolutionary significance in this type of nuclear reorganization. Absence of effective interbreeding between a new form and its immediate ancestors is implied if gain or loss of odd chromosomes is to be permanent. It is not impossible that the cytological change itself may be a causal factor to this genetical isolation, and it is not unreasonable

<sup>1</sup> *Aneuploidy* is the possession by related forms of numbers which are *not* multiples of each other.

*Polyploidy*, as here used, denotes the possession by related forms of chromosome numbers which are simple multiples of each other (and integral multiples of a common number, the 'fundamental'). 'Allopolyploidy' and 'autopolyploidy' are not distinguished.

to regard such isolation as one of the circumstances by which such a new form may achieve generic rank. Whatever the mechanism, however, the above fact suggests that new species may not all be equipotential as evolving units, and that evolution should not be regarded as a single continuous process synonymous with 'Origin of Species'. Multiplication of specific forms may represent nothing more far reaching than a local elaboration of detail.<sup>1</sup> It is the *successive* origin of genera (or of whatever large systematic unit that may prove to correspond to the natural step) that alone seems to constitute *progressive evolution*, and in this *aneuploidy would appear to be a positive factor*.

Whether the aneuploid species of, for instance, the Brassicinae are really such incipient genera (which implies that here, if anywhere in the family, the new genera of the future are arising) cannot yet be answered. It is always possible that the chromosome as an evolutionary factor may have a different significance in this group from that deduced for the rest of the family in the past. On the other hand, the possibility makes the particular group of special interest, and the author would welcome wild material of any members of it.

*Polyploidy*, unlike aneuploidy, is rarely an intergeneric relationship, but is widespread as an interspecific (and intra-specific<sup>2</sup>) relation throughout the family, the most complete series being that of *Aethionema*.

High polyploid numbers ( $8x$  and  $9x$ ) have been found, notably in *Crambe*, *Erysimum*, and *Lepidium*, the octoploidy in *Crambe* corresponding to an actual complement of 120 chromosomes. In comparison with this the lowness of the fundamental numbers of these same genera is striking, *Erysimum* and *Lepidium* being on 8, *Crambe* on 15, and, moreover, this 15 is among the highest fundamental numbers yet established for any genus, being exceeded only by the 17 of *Campanula* (Marchal (35)), 19 in the Salicaceae (Blackburn and Harrison (2)), and 23 in *Pyrola* (Hagerup (13)). This striking *absence of high numbers in generic fundamentals*, in contrast to the condition obtaining in species, is as suggestive a fact as is the relative distribution of aneuploidy and polyploidy among species and genera.

Experimental cytogenetical evidence has shown clearly the importance of polyploid changes, notably chromosome doubling, in stabilizing new forms of hybrid or mutant origin, e.g. *Raphano-Brassica* (Karpechenko (24)), *Primula Kewensis* (Digby (9)). The prevalence of polyploidy among the natural species of genera makes these experiments of especial interest. Furthermore, instances are multiplying in which the same factors known in

<sup>1</sup> This same probability has been clearly suggested in groups other than the Cruciferae, e.g. Heitz (18): 'dass in allen diesen Fällen' (Papaveraceae, Ranunculaceae, Onagraceae, Scrophulariaceae) 'die Bildung von Gattungen auf ganz andere Weise erfolgt, als die Bildung 'der Arten', oder, mit anderen Worten, dass die Artbildung im eigentlichen Sinne (etwa so wie sie sich Baur bei *Antirrhinum* vorstellt) über die Gattungsgrenzen nicht hinauskommt.'

<sup>2</sup> See *Nasturtium*, *Crambe*, &c.



the experimental garden can be demonstrated to have taken part in the natural formation of Linnean species, e.g. *Rosa* (Täckholm (51)), *Ochna* (Chiarugi and Francini (7)). But this cannot be the whole evolutionary significance of polyploidy. The complete absence of any fundamental numbers reaching anywhere near the order of, for instance, 119 or 73 (which would be comparable in magnitude to actual specific numbers found, i.e. 120 and 72) indicates that in the Cruciferae no high polyploid as such has ever given rise to a new generic type. On the other hand, though many ways are known in which multiplication of chromosomes may occur (by failure to separate at mitosis or meiosis, &c.),<sup>1</sup> the effective reversal of this has never yet been seen. The conclusion seems inevitable that, in contrast to an aneuploid change, *high polyploidy*, however much it may figure in elaboration of specific forms, is a *barrier to true progress*.

*Conclusions.* The general survey suggests that the present distribution of chromosome numbers in the family is only logically explicable on the assumption that there have been two independent types of evolutionary activity which, in general terms, have respectively resulted in the initiation of new 'species' and new 'genera'. The cytological changes accompanying the genesis of these two categories of taxonomic units are found, on the whole, to be different in kind. It is difficult to avoid the conclusion, not only that nuclear reorganizations have formed some part of the mechanism of evolution, but that they have contributed both positive and negative factors in the path of true progress.

As a final corollary it is perhaps not unprofitable to point out that any influence of the mechanical distribution of the chromosomes upon the course of evolution must be exercised without direct relation to the degree of 'adaptedness' or 'advance' in the morphology of the plant as a whole.

#### SUMMARY.

1. A general cytological survey of the Cruciferae has been attempted in order to ascertain, so far as possible, the effective evolutionary trends in nuclear organization in a recent natural group, and to correlate the chromosomes with taxonomy. Root-tips of 250 odd species, representing some 80 genera, have been available.

2. The chromosomes are usually small, exceptions being in *Matthiola*, *Hesperis*, *Iberis*, *Bunias*, and *Menonvillea*.

3. Supernumerary fragments are recorded in species of *Iberis*.

4. Local vegetative polyploidy has been recorded in roots of 8 species.

5. An *aneuploid* relationship is frequent between the fundamental numbers of *genera*, though it is rare between the species of the same genus. In the Brassicinae, however, and, to a lesser extent, in the Hesperidinae,

<sup>1</sup> See 27, 29, 39, 42, &c.

it is the rule. The condition in *Iberis* is exceptional. On the whole, aneuploid loss is the more frequent, and has been demonstrated very clearly in *Matthiola*. *Biscutella* presents the converse condition.

6. Polyploidy between species is frequent, the highest values being recorded in *Crambe*.

7. Fundamental numbers 5, 6, 7, 8, 9, 11, 13, and 15 have been recorded, but nothing higher, although the actual somatic numbers in some species of *Crambe* reach 120.

8. It is suggested, from the evidence presented in connexion with the last three paragraphs, that there have been two distinct evolutionary processes in the Cruciferae—Multiplication of Forms and Progressive Evolution. Aneuploidy appears to be frequently a positive factor in the latter process. Polyploidy, on the other hand, though closely involved in the former, appears often to be an insuperable barrier to true progress.

9. The principal problems reserved for further study are the peculiar chromosomal changes observed in *Nasturtium* and *Biscutella*.

#### *Summary of Positive Taxonomic Conclusions.*

10. The cytological evidence emphasizes the desirability of separating the following genera:

*Eunomia* from *Aethionema*.  
*Sisymbrella* from *Sisymbrium*.  
*Aarmoracia* from *Cochlearia*.  
*Lesquerella* from *Vesicaria*.

11. A closer affinity is suggested between the following genera:

*Cossonia* and *Morisia*.  
*Teesdalia* and the *Capsellinae*.

12. *Hutchinsia procumbens* should definitely be named thus, and not as a *Capsella* (e.g. *Capsella procumbens* in Index Kewensis).

13. Further cytological work would elucidate systematic problems in:

*Brassicinae*.  
*Lesquerella*.  
*Arabidopsis*.

14. Systematic revision in the light of cytology seems desirable in:

*Iberis*.  
*Matthiola*.  
*Malcolmia*.

15. The principal emendations of von Hayek's system introduced by O. E. Schulz in *Das Pflanzenreich* are supported.

## LIST OF CHROMOSOME NUMBERS.

The specific nomenclature is that of the Index Kewensis, except where otherwise stated.

An asterisk beside a species denotes that the plant used was known to be wild or to be from seed of a wild plant.

The numbers immediately following the specific names refer to the sources of material listed on p. 552.

An entry in square brackets indicates a report which is considered by the present writer to be erroneous.

The numbers in the right hand column refer to the literature list. A dash in this column means that the data are solely the result of the present work. The initials I. M. accompany original observations where these are additional to those of other writers on the same species.

*N.B.—This list supersedes and, in places, corrects the preliminary report submitted to Professor Tischler, and published by him (54) in Tabulae Biologicae Periodicae.*

## Tribus. ARABIDEAE.

Subtribus. *Sisymbriinae*.

## SISYMBRIUM (46).

	2 n.	n.	Ref. No.
[ <i>S. strictissimum</i> . . . . .]	—	16]	(28)
[ <i>S. supinum</i> , L. . . . .]	—	16]	(22)
<i>S. altissimum</i> , L. 9 . . . . .	14	—	—
<i>S. Assoanum</i> Loscos and Pardo, 24 <i>a</i> . . . . .	14	—	—
<i>S. austriacum</i> , Jacq. 9 . . . . .	14	—	—
<i>S. pyrenaicum</i> , (L.) Vill. 21 . . . . .	14	—	—
<i>S. wolgensse</i> , Marsch B. 9 . . . . .	14	—	—
<i>S. polyceratium</i> , L. 9 . . . . .	prob. 28	—	—
<i>S. strictissimum</i> , L. 24 <i>a</i> . . . . .	28	—	—
<i>S. runcinatum</i> , Lag. 9. 20 . . . . .	42	—	—

## DESCURAINIA (46).

<i>D. Cumingiana</i> , (Fisch & Mey) Prantl. 9 <i>a</i> . . . . .	14	—	—
<i>D. myriophylla</i> , (Willd.) R. E. Fries 24 <i>a</i> . . . . .	14 (locally 28)	—	—
<i>D. Menziesii</i> , (DC.) O. E. Schulz (= <i>pin-nata</i> , Greene) 70 . . . . .	28	—	—
<i>D. sophia</i> , (L.) Webb 9 . . . . .	28 (locally 56)	—	—

Subtribus. *Erysiminiæ*.

## ERYSIMUM.

<i>E. canescens</i> , Roth. 45 <i>a</i> . . . . .	72	—	—
<i>E. rupestre</i> , DC. 24 <i>a</i> . . . . .	ca. 56	—	—
<i>E. helveticum</i> . . . . .	—	24	(21)
<i>E. sylvestre</i> . . . . .	—	ca. 24	"
<i>E. purpureum</i> , Auch ex Boiss. 24 <i>a</i> . . . . .	ca. 40	—	—
<i>E. Perowskianum</i> , Fisch & Mey 9 <i>a</i> . . . . .	32-36	—	—
<i>E. hieraciifolium</i> . . . . .	prob. 32	—	(21)
<i>E. ochroleucum</i> . . . . .	—	prob. 16	"
<i>E. cheiranthoides</i> , L. 6 . . . . .	16 I.M.	8	"
<i>E. 'cuspidatum</i> , DC.' 9 <i>a</i> . . . . .	16	—	—
<i>E. repandum</i> , L. 9 . . . . .	14-16	—	—
<i>E. thyrsoides</i> , Boiss. var. <i>alpinum</i> 24 <i>a</i> . . . . .	prob. 16	—	—
<i>E. linifolium</i> , J. Gay 5 . . . . .	14	—	—

Subtribus. *Cardamininae*.

## BARBAREA (16).

	2 n.	n.	Ref. No.
B. stricta, Fries. 9 . . . . .	16-18	—	—
B. intermedia, Bor. 3. 9 . . . . .	16	—	—
B. minor, L. 2 a . . . . .	16	—	—
B. rupicola, Moris 30 . . . . .	16.	—	—
B. verna, (Mill) Aschers 24 a . . . . .	16	—	—
B. vulgaris, R.Br. 21 . . . . .	16,	—	—

## RORIPA (16).

R. austriaca, (Crantz) Besser 9 a . . . . .	16	—	—
*R. pyrenaica, L. 30. 66 . . . . .	16	—	—
R. sylvestris, (R.Br.) Besser 34 . . . . .	32	—	—

## ARMORACIA (16).

A. lapathifolia, Gilib. (Cochlearia Armo- racia) 49 . . . . .	32	—	—
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## NASTURTIUM.

*N. officinale, R.Br. . . . .	32, 48, and 64	—	—
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## CARDAMINE (48).

Sectio 1. *Dentaria*.

C. polyphylla, (W.K.) O. E. Sch. . . . .	48	—	(49)
C. digitata, (Lam.) O. E. Sch. (= penta- phylla) . . . . .	48	—	"
C. pinnata, (Lam.) R.Br. . . . .	48	—	"
C. bulbifera, (L.), Cranz . . . . .	96?	—	"
C. savensis, O. E. Sch. 13 . . . . .	80 at least	—	—

Sectio 2. *Eutrechtophyllum*.

C. californica, (Nutt) Green 24 a . . . . .	32	—	—
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Sectio 4. *Coriophyllum*.

C. trifolia, L. 2 a . . . . .	16	—	—
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Sectio 5. *Macrophyllum*.

C. leucantha (T.) O. E. Sch. prol. yezo- ensis (Max.) O. E. Sch. 13 . . . . .	46-48	—	—
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Sectio 8. *Eucardamine*.

*C. amara, L. 5. 58. 61 . . . . .	16	—	—
C. asarifolia, L. 5 . . . . .	16	—	—
*C. hirsuta, L. 52 . . . . .	16	—	—
C. impatiens, L. 9 . . . . .	16	—	—
*C. flexuosa, With. 51 . . . . .	ca. 32	—	—
*C. pratensis, L. 52 . . . . .	ca. 32 <sup>1</sup>	—	—
2 'double' sterile vars. 57. 5 . . . . .	ca. 64	—	—
C. chenopodiifolia, Pers. 9 a . . . . .	64	—	—

<sup>1</sup> In a paper by W. T. C. Lawrence on 'The chromosome constitution of *Cardamine pratensis* and *Verbascum phoeniceum*' (Journ. of Gen. XIII, p. 183, 1931), which has appeared while this work was in the press the somatic numbers for *C. pratensis*, *C. asarifolia* and *C. amara* have been definitely determined as 30, 14 and 16 respectively. Fusion of chromosomes is considered to underlie the aneuploidy of the first two species.

	2 n.	n.	Ref. No.
Sectio 9. <i>Cardaminella</i> .			
C. alpina, Willd. 16 . . . . .	16	—	—
C. Plumierii Vill. 34 . . . . .	16	—	—
C. resedifolia, L. 16 . . . . .	16	—	—
Sectio 10. <i>Pteroneurum</i> .			
C. graeca, L. 9a . . . . .	16	—	—
Sectio 11. <i>Spirolobus</i> .			
C. chelidonia, L. 37 . . . . .	16	—	—
CARDAMINOPSIS.			
C. Halleri, L. . . . .	—	8	(21)
ARABIDOPSIS.			
A. thaliana, (L.), Heynh. . . . .	—	5	(23), (21) (59)
*A. pumila, (Steph.) Busch. 69 . . . . .	32	—	—
A. suecica, (Fries) Norrlin. 9a . . . . .	26-8	—	—
TURRITIS.			
T. glabra, L. 2a . . . . .	16 I.M.	16	(21)
Subtribus. <i>Arabidinae</i> .			
ARABIS			
7 species . . . . .	—	8	(21)
1 „ . . . . .	16	—	„
2 „ . . . . .	—	16	„
AUBRIETIA.			
4 species . . . . .	—	8	(21)
Subtribus. <i>Isatidinae</i> .			
MYAGRUM.			
M. perfoliatum, L. . . . .	—	7	(22)
ISATIS (16).			
I. tinctoria, L. var. vulgaris Koch. ('alep- pica' <sup>1</sup> ) 9 . . . . .	28	—	—
I. tinctoria var. vulgaris f. sativa, D.C. 46 . . . . .	28	—	—
*I. tinctoria var. vulgaris sub var, Taurica Alef 75 . . . . .	28	—	—
I. tinctoria var. praecox Koch. ('cane- scens' <sup>1</sup> ) 12 . . . . .	28	—	—
I. tinctoria var. canescens, Gren. & God. ('brachycarpa' <sup>1</sup> ) 15 . . . . .	28	—	—
TAUSCHERIA.			
T. lasiocarpa, Fisch. . . . .	14	—	—
Subtribus. <i>Buniadinae</i> .			
BUNIAS.			
B. erucago, L. 45a . . . . .	14 I.M.	7	(21)
B. orientalis, L. 9. 24. . . . .	14	—	(19), (14)
	42	7	(21)

<sup>1</sup> The name under which the seed was received.

	2 n.	n.	Ref. No.
GOLDBACHIA.			
G. laevigata, DC. 9.	28 I.M.	14	(22)
*G. laevigata, var. ascendens Boiss., (G. torulosa, DC.) 69	28	—	—

*Genera separated from von Hayek's genera by Schulz (46).*

HUGUENINIA (formerly <i>Sisymbrium</i> ).			
H. tanacetifolia, (L.) Reich. 34	14-16	—	—
SISYMBRELLA (formerly <i>Sisymbrium</i> ).			
*S. aspera, (L.) Spach 60	16	—	—
S. dentata, (L.) O. E. Sch. 9 a.	32	—	—

*Recent additional genera (46).*

ONURIS.			
*O. graminifolia, Phil. 71	18	—	—
XERODRABA.			
*X. pycnophylloides, (Speg.) Skotts. 71	22	—	—

Tribus. *ALYSSEAE*.

Subtribus. *Hesperidinae*.

CHEIRANTHUS.			
C. cheiri, L.	—	7	(21)
*C. cheiri, var. fruticosus 63	14	—	—
*C. cinereus, Webb. & Berth. 67	28	—	—
C. Menziesii, B. & H. 13	28	—	—
*C. tenuifolius L'Herit. 68	prob. 28	—	—
C. Allionii, Hort. 24 a	40-42	—	—
C. mutabilis, L'Herit. 13	prob. 42	—	—

HESPERIS.

[H. matronalis . . . . .	—	14]	(21)
[H. tristis . . . . .	—	14]?	"
H. bicuspidata, Willd. 11	14	—	—
H. Steveniana, DC. 19	14	—	—
H. tristis, L. 22	14	14	(21)
H. matronalis, L. 45 a. 20. 5. 50	24	14	—
H. matronalis, var. lutea 42	24	—	—
H. runcinata, W. & K. 24 a	24	—	—
H. sylvestris, Crantz. 41	26	—	—
H. lutea, Max. 9. 34	28	—	—

MALCOLMIA.

M. maritima, R.Br. 13	14-16 I.M.	7	(21)
*M. africana, R.Br. 32	28	(7)?	"
M. chia, DC. 31	32	—	—
M. littorea R.Br. 9 a. 24 a.	20	ca. 10	—

MATTHIOLA.

M. incana, R. Br.	16, 14	7	(1), (11), (21), (31)
M. bicornis, DC.	14	7	(22), (34)
M. fenestralis, R. Br.	14	—	—
M. parviflora, R. Br.	14	—	(34)

	2n.	n.	Ref. No.
M. sinuata, R. Br. . . . .	14	7	(22), (34)
M. sinuata glabra, var. albiflora . . . . .	—	7	(34)
M. odoratissima, R. Br. . . . .	12	—	(22)
M. tatarica, DC. . . . .	12	—	(22)
M. thessala, Boiss & Orph. . . . .	12	6	(22)
M. tristis, R. Br. . . . .	—	6	(22), (34)
M. Valesiaca, J. Gay. . . . .	12	6	

Subtribus. *Brayinae*.

## BRAYA.

B. alpina, Sternb. & Hoppe. 2a . . . . .	32	—	—
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## CHORISPORA.

C. tenella, (Pall) DC. 45 a . . . . .	14 I.M.	7	(22)
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Subtribus. *Euclidiinae*.

## EUCLIDIUM.

*E. tataricum, DC. 69 . . . . .	14	—	—
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Subtribus. *Lunariinae*.

## RICOTIA.

R. lunaria, DC. 9 . . . . .	28	—	—
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## LUNARIA.

L. annua, L. 45 . . . . .	apparently 30 (poss. 28)	—	—
L. rediviva, L. 46 a . . . . .	"	—	—

Subtribus. *Alyssinae*.FIBIGIA (= *Farsetia*, Index Kewensis).

F. clypeata, R. Br. 34 . . . . .	16	—	—
F. eriocarpa, DC. 43 . . . . .	14-16	—	—

## BERTEROA.

B. incana, L. 24 a . . . . .	16	—	—
*B. mutabile, Vent. 69 . . . . .	16	—	—

## ALYSSUM.

A. calycinum, L. 9 a . . . . .	32 I.M.	16	(21)
A. pyrenaicum, Lap. 24 a . . . . .	32	—	—
A. repens, Baum. 24 . . . . .	32	—	—
A. spinosum, L. 24 a . . . . .	32	—	—
A. thessalum, Hal. 1 . . . . .	32	—	—
—A. Wulfenianum, Bernh. 16 . . . . .	32	—	—
A. maritimum, Desv. 9 a . . . . .	24 I.M.	12	(21)
A. 'Arduini', Fritsch., 48 . . . . .	16	—	—
A. argenteum . . . . .	16	—	(28)
A. Borzaeanum, Nyarady 7 . . . . .	16	—	—
A. campestre, L. 43 . . . . .	16	—	—
—A. corymbosum, Grieseb. . . . .	—	8	(21)
A. cuneifolium, Ten 2 a . . . . .	16	—	—

ALYSSUM (*continued*).

	2 n.	n.	Ref. No.
A. dasycarpum, Stev. 5 . . . . .	16	—	—
A. Doerfleri 24 a . . . . .	16	—	—
A. edentulum, Walldst. & Kit. 23 . . . . .	16 I.M.	8	(21)
A. linifolium, Steph. 9 a. 13 . . . . .	14-16	—	—
A. Moellendorffianum, Asch. 2 a . . . . .	16	—	—
A. montanum, L. 1. 9 . . . . .	16	—	—
A. murale . . . . .	—	8	(21)
A. orientale, Ard. 47 . . . . .	16	—	—
A. ovirense, A. Kern. 22 . . . . .	16	—	—
A. rostratum, Stev. 40 . . . . .	16	—	—
A. saxatile, L. . . . .	16	8	(21), (28)
A. tortuosum, Rupr. 31 . . . . .	16	—	—
*A. transsylvanicum, Schur. . . . .	16	—	—
A. Wierzbickii . . . . .	16	—	(28)

## VESICARIA.

V. graeca, Reut. 13 . . . . .	16	—	—
V. utriculata, DC. 9. 46 a . . . . .	16	—	—

## THYSANOCARPUS.

T. curvipes, Hook. 9 a . . . . .	28	—	—
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## CLYPEOLA.

C. Jonthlaspi, L. . . . .	—	16	(21)
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Subtribus. *Drabinae*.

## SCHIEVERECKIA.

S. Bornmuelleri, Prantl. 2 a . . . . .	16	—	—
S. podolica, Bess. 2 a . . . . .	16	—	—

DRABA<sup>1</sup>.

3 species . . . . .	—	8	(17)
1 species . . . . .	—	16	—
1 species . . . . .	—	24	—
2 species . . . . .	—	32	—
D. cacuminum . . . . .	—	ca. 30	—
D. magellanica . . . . .	—	24, 32, 40	—

## EROPHILA.

E. verna, vars. . . . .	—	7	(59, 60)
	—	15	—
	—	32	—

Tribus. *BRASSICEAE*.Subtribus. *Brassicinae*.

## DIPILOTAXIS (44).

*D. erucoides, (L.)DC. 46. 65 . . . . .	14	—	—
*D. catholica, (L.)DC. 62 . . . . .	18	—	—
D. viminea, (L.)DC. 24 a . . . . .	ca. 20	—	—
*D. tenuifolia, (L.)DC. 27. 56 . . . . .	22	—	—

<sup>1</sup> See, further, Tischler (54).



	2 n.	n.	Ref. No.
BRASSICA (44).			
Sectio. <i>Brassicotypus</i> .			
B. 'Napella Chaix' . . . . .	—	19	(36)
B. Napus, L. . . . .	36 <sup>2</sup>	18	"
B. juncea, Czern . . . . .	36 <sup>2</sup>	18	"
*B. elongata, Ehrf. 69 . . . . .	22 I.M.	—	—
B. campestris Ehrf. . . . .	20 <sup>2</sup>	—	(33)
B. oleracea, L. . . . .	18 <sup>2</sup>	9	"
B. cernua, Forbes and Hem. . . . .	18 <sup>2</sup>	9	"
*B. balearica, Pers. 65 . . . . .	18	—	—
B. insularis, Moris 32 . . . . .	18	—	—
*B. rupestris, Raf. Carall. 65 . . . . .	18	—	—
Sectio. <i>Brassicaria</i> .			
Sectio. <i>Melanosinapis</i> .			
B. nigra, (L) Koch . . . . .	16 <sup>2</sup>	—	—
SINAPIS (44).			
S. alba . . . . .	24	—	(23)
S. dissecta . . . . .	24	—	"
S. arvensis . . . . .	18	—	"
*S. pubescens, L. 65 . . . . .	18	—	—
ERUCA.			
E. sativa, Gars. 27. 45 <sup>a</sup> . . . . .	22	—	—
*E. 'cappadocica', Reut. 69 . . . . .	22	—	—
ERUCASTRUM (44).			
E. abyssinicum, (A. Rich). O.E. Sch. 45 <sup>a</sup> . . . . .	32	—	—
E. nasturtiifolium, (Poi) O.E. Sch. 45 <sup>a</sup> . . . . .	32	—	—
E. elatum, (Ball) O.E. Sch. 39 . . . . .	30	—	—
*E. gallicum, (Willd.) O.E. Sch. 9 a 74 . . . . .	30	—	—
E. arabicum, Fisch & Mey. 9 . . . . .	32-30	—	—
Subtribus. <i>Raphaninae</i> .			
REBOUDIA.			
R. erucarioides, Coss & Dur. (= Erucaria aleppica, Gaertn.) 31 . . . . .	16	—	—
MORISIA.			
M. hypogaea, J. Gay. 24 <sup>a</sup> . . . . .	14	—	—
RAPISTRUM.			
R. rugosum, All 20. 46 . . . . .	16	—	—
CAKILE.			
C. maritima, Scop. 24 <sup>a</sup> . . . . .	18 I.M.	9	(22)

<sup>1</sup> See Bibliographia genetica V. I, (33).

	2n.	n.	Ref. No.
CRAMBE (44).			
*C. fruticosa, L. 68 . . . . .	30 (locally 60)	15	—
C. filiformis, Jacq. 9a . . . . .	30	—	—
C. orientalis, var. koktebelica (Junge) 33 . . . . .	30	—	—
C. hispanica, L. 46 a 16 . . . . .	60	—	—
C. maritima, L. 46 a . . . . .	60	—	—
C. tatarica, var. pinnatifida, (R. Br.) 46 a 60 (locally 120)	—	—	—
C. abyssinica, Hoch. 29 . . . . .	90	—	—
C. cordifolia, Stev. 13 . . . . .	ca. 120	—	—
C. grandiflora, DC. 10 . . . . .	ca. 120	—	—
C. orientalis, var. juncea, (Marsch B.) 13 . . . . .	ca. 120	—	—
C. tatarica, Seb. 32 . . . . .	ca. 120	—	—
CALEPINA (44).			
C. irregularis, (Asso.) Thell. (= C. corvini)			
9a . . . . .	14 I.M.	21	(22)
RAPHANUS (44).			
*R. maritimus, Smith 53 . . . . .	18	—	—
R. raphanistrum . . . . .	18	9	(24)
R. sativus . . . . .	18	9	—
R. 'caudatus' 46 a . . . . .	18	—	—
*R. Landra, Morelli 64 . . . . .	18	—	—
COSSONIA (= <i>Raffenaldia</i> ) (44).			
C. africana, Dur. (= <i>Raffenaldia</i> primu- loidea) 24 a . . . . .	14	—	—
Subtribus. <i>Vellinae</i> .			
CARRICHTERA.			
C. 'vella, DC' 9 . . . . .	16 I.M.	8	(22)
C. 'annua, (L.) Aschers' 5 . . . . .	32 I.M.	8	"
SUCCOWIA.			
S. balearica, Medic. 28. 43 . . . . .	36 I.M.	[16]	(22)
Subtribus. <i>Moricandiinae</i> .			
CONRINGIA.			
C. orientalis . . . . .	—	7	(22)
C. planisiliqua, (Fisch & Mey) O.E. Sch. 6 . . . . .	prob. 14 I.M.	—	—
MORICANDIA.			
M. arvensis, DC. 30 . . . . .	28	—	—
ORYCHOPHRAGMUS.			
O. violaceus (L.) O.E. Sch. 24 a . . . . .	24	—	—
Tribus. <i>LEPIDIAE</i> .			
Subtribus. <i>Lepidiinae</i> .			
LEPIDIUM (53).			
Sectio. <i>Cardaria</i> .			
*L. Draba, L. 9 a 76 . . . . .	64	—	—

	2 n.	n.	Ref. No.
Sectio. <i>Lepia</i> .			
* <i>L. heterophyllum</i> , Benth. (= <i>L. Smithii</i> , Hook) 53. 24 . . . . .	16	—	—
<i>L. hirtum</i> , (L.) DC. var. <i>calycotrichum</i> , Kunz. 26 . . . . .	16	—	—
Sectio. <i>Cardamon</i> .			
<i>L. sativum</i> . L. . . . .	—	8	(22)
Sectio. <i>Nasturtioides</i> .			
<i>L. cartilagineum</i> (J. Mey), Thell., var. <i>crassifolium</i> , W. K. 20 . . . . .	40	—	—
<del><i>L. densiflorum</i></del> , Schrad. 9 . . . . .	32	—	—
<i>L. pubicarpum</i> , A. Nels. 13 . . . . .	32	—	—
<i>L. ruderales</i> , L. 43, &c. . . . .	prob. 32	—	—
<i>L. virginicum</i> , L. 9 . . . . .	32	—	—
<i>L. latifolium</i> , L. 27. 37 . . . . .	prob. 24	—	—
<i>L. Alluaudii</i> , Maire 39 . . . . .	15-16	—	—
<i>L. Armoracium</i> , F. & M. 45 . . . . .	16	—	—
<i>L. graminifolium</i> , L. 9 <sup>a</sup> . . . . .	16	—	—
<i>L. perfoliatum</i> , L. 9 <sup>a</sup> . . . . .	16	—	—
<i>L. reticulatum</i> , How. 9. 34, &c. <sup>1</sup> . . . . .	16	—	—
* <i>L. vesicarium</i> , L. 69 . . . . .	16 (locally 32)	—	—
HYMENOPHYSA.			
<i>H. 'pubescens'</i> 38 . . . . .	24	—	—
CORONOPUS.			
<i>C. procumbens</i> ( <i>Senebiera Coronopus</i> , Poir.) 9 <sup>a</sup> . 45 . . . . .	32	—	—
* <i>C. didymus</i> ( <i>Senebiera pinnatifida</i> , DC.) 9 <sup>a</sup> . 45. 55 . . . . .	32	—	—
BISCUTELLA.			
Sectio. <i>Iondraba</i> .			
<i>B. auriculata</i> , L. 45 . . . . .	16 I.M.	8	(22)
<i>B. auriculata</i> , L. var. <i>erigerifolia</i> , DC. 45 . . . . .	16	—	—
Sectio. <i>Thlaspidium</i> (32).			
<i>B. apula</i> , L. ( <i>B. didyma</i> , L.) 9 . . . . .	16	—	—
<i>B. ciliata</i> , DC. 39 . . . . .	16	—	—
* <i>B. lyrata</i> , L. 65 . . . . .	ca. 16	—	—
<i>B. glacialis</i> , Jord. 24 <sup>a</sup> . . . . .	18	—	—
* <i>B. laevigata</i> , L. 9 <sup>a</sup> 74 . . . . .	18 and 36	—	—
Subtribus. <i>Iberidinae</i> .			
HUTCHINSIA (16).			
<i>H. alpina</i> , (L.) R. Br. 46 <sup>a</sup> . . . . .	12	—	—
* <i>H. petraea</i> , (L.) R. Br. 45 <sup>a</sup> . 54 . . . . .	12	—	—
* <i>H. procumbens</i> , (L.) Desv. (= <i>Capsella procumbens</i> ) 9 <sup>a</sup> . 65 . . . . .	12 (locally 24)	—	—

<sup>1</sup> Commonly in cultivation as '*L. Menziesii*' or '*L. Humboldtii*'.

	2 n.	n.	Ref. No.
<b>IBERIS.</b>			
( <i>I. pinnata</i> . . . . .)	16)	—	(28)
<i>I. Jordani</i> , Boiss. 24 <i>a</i> . . . . .	22	—	—
<i>I. nana</i> , All. 13 . . . . .	22	—	—
<i>I. Pruiti</i> , Tineo. 3. 13. 45 <i>a</i> . . . . .	22	—	—
* <i>I. semperflorens</i> , L. 65 . . . . .	22 (locally 44)	—	—
<i>I.</i> „ „ var. <i>garrexiana</i> 24 <i>a</i> . . . . .	22 + 1 fragment	—	—
<i>I. correaefolia</i> , Hort. 24 <i>a</i> . . . . .	44	—	—
<i>I. Garrexiana</i> , All. 45 <i>a</i> . . . . .	44	—	—
<i>I. saxatile</i> , L. 9 <i>a</i> . 24 <i>a</i> . . . . .	apparently 22 + 2 fragments ca. 50 (= 44 fragmented?)	—	—
<i>I. corifolia</i> , Sweet. 13 . . . . .	—	—	—
<i>I. affinis</i> , Jord. 18 . . . . .	14	—	—
<i>I. amara</i> , L. 17. 35 . . . . .	14	—	—
<i>I. gibraltarica</i> , L. 24 <i>a</i> . . . . .	14	—	—
<i>I. Lagascana</i> , DC. 9 . . . . .	14	—	—
<i>I. odorata</i> , Boiss. 9. 9 <i>a</i> . . . . .	14	—	—
<i>I. pectinata</i> , Boiss. 9 . . . . .	14	—	—
<i>I. taurica</i> , DC. 9 <i>a</i> . 24 <i>a</i> . . . . .	14	—	—
<i>I. umbellata</i> , DC. 45 . . . . .	14	—	—
<b>AETHIONEMA.</b>			
<i>A. cordatum</i> , Boiss. 13 . . . . .	60	—	—
<i>A. amoenum</i> , Hort. 24 <i>a</i> . . . . .	48	—	—
<i>A. armenum</i> , Boiss. 24 <i>a</i> . . . . .	48	—	—
<i>A. diastrophis</i> , Bunge. 3. 19 . . . . .	48	—	—
<i>A. grandiflorum</i> , Boiss. & Hohen. 2 <i>a</i> . . . . .	48	—	—
<i>A. pulchellum</i> , Boiss. & Hohen. 16 . . . . .	48	—	—
<i>A. saxatile</i> , R. Br. 9 <i>a</i> . . . . .	48	—	—
<i>A. persicum</i> , Hort. 16 . . . . .	36	—	—
<i>A. Thomasianum</i> , J. Gay. 5 . . . . .	36	—	—
<i>A. coridifolium</i> , DC. 24 <i>a</i> . . . . .	24	—	—
<i>A. graecum</i> , Boiss. & Spreng. 24 <i>a</i> . . . . .	24	—	—
<i>A. schistosum</i> , Boiss. & Kotsch. 24 <i>a</i> . . . . .	24	—	—
* <i>A. arabicum</i> , (L.) Andr. 9. 69 . . . . .	22-24	—	—
<i>A. cristatum</i> , DC. 9 . . . . .	22-24	—	—
Subtribus. <i>Thlaspidinae</i> .			
<b>COCHLEARIA.</b>			
<i>C. alpina</i> . . . . .	28	—	(8)
<i>C. officinalis</i> . . . . .	28	—	—
<i>C. anglica</i> . . . . .	37-42	—	—
<i>C. danica</i> . . . . .	42	—	—
<i>C. micacea</i> . . . . .	34-36	—	—
<i>C. glastifolia</i> , L. 13 . . . . .	38 I.M.	—	—
<b>COLUTEOCARPUS.</b>			
<i>C. reticulatus</i> , Boiss. 24 <i>a</i> . . . . .	14	—	—
<b>IONOPSIDIUM.</b>			
<i>I. acaule</i> , Rchb. . . . .	24	12	(6)
<i>I. Savianum</i> , (Cav.) Ball. . . . .	32	16	—
<b>EUNOMIA (= <i>Aethionema</i> in Index Kewensis).</b>			
<i>E. iberidea</i> , Boiss. 24 <i>a</i> . . . . .	28	—	—
<i>E. oppositifolia</i> , DC. 13. 24 <i>a</i> . . . . .	14	—	—

	2 n.	n.	Ref. No.
THLASPI.			
T. montanum, L. 9 . . . . .	prob. 28	—	—
T. alpestre, L. sensu lato. 10 . . . . .	14	—	—
T. arvensis, L. 9 . . . . .	14	—	—
T. brevistylum, Jord. 34 . . . . .	14	—	—
T. cepeaeifolium, (L.) Gaud. 24 a . . . . .	14	—	—
T. ceratocarpon, Murr. 9 . . . . .	14 I.M.	7	(22)
T. cilicicum, Boiss. 45 a . . . . .	14 (locally 28)	—	—
T. densiflorum, B. H. 7 . . . . .	14	—	—
T. 'jankae' 24 a . . . . .	14-	—	—
*T. Kowatsii, Heuff. 66 . . . . .	14-	—	—
T. limosellifolium, Reut. 5 . . . . .	14-	—	—
T. praecox, Wulf. 29. 34 . . . . .	14-	—	—
T. rotundifolium, (L.) Gaud. 13 . . . . .	14-	—	—
T. stylosum, Nym. 44 . . . . .	14.	—	—

## TEESDALIA.

T. nudicaulis, R. Br. 14 . . . . .	36	—	—
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## PELTARIA.

P. alliacea, Jacq. 45 a . . . . .	28 (locally 56)	—	—
P. turkmena, Lipsky. 13 . . . . .	14	—	—

Subtribus. *Capsellinae*.

CAMELINA (Names are those under which the plants were received).

C. sativa, Cr. 45 . . . . .	40	—	—
C. glabrata, (DC.) Fritsch. 15. 46 . . . . .	40	—	—
C. alyssum, Thell. 45 . . . . .	40 I.M.	[21]	(21)
C. foetida, 46 a . . . . .	40	—	—
C. dentata 5 . . . . .	40	—	—
C. linicola, Schimp. & Sp. 9 a . . . . .	40	—	—
*C. microcarpa, Andr. 45. 69 . . . . .	40	—	—

## CAPSELLA.

C. bursa pastoris . . . . .	32	16	(40), (28)
C. Heegeri . . . . .	—	16	(35)
C. djurdjurae . . . . .	—	16	(20)
C. grandiflora, Boiss. 9 a . . . . .	16 I.M.	8	"
C. occidentalis . . . . .	—	8	"
C. orientalis . . . . .	—	8	"
C. penarthae . . . . .	—	8	"
C. rubella . . . . .	—	8	"
C. tuscaloosae . . . . .	—	8	"
C. Viguieri . . . . .	—	8	"

VOGELIA (= *Neslia*).

V. paniculata, Desv. 46 a . . . . .	14 I.M.	7	(21)
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Tribus. *SCHIZOPETALEAE*.Subtribus. *Physariinae*.

## LESQUERELLA (37).

L. argentea, (Push) MacMillan. 13 . . . . .	10	—	—
L. gracilis, (Hook) Wats. 9 a. 9 . . . . .	12	—	—
L. grandiflorum, (Hook) Wats. 9 a. 9 . . . . .	18	—	—
*L. 'mendocina, (Phil) Kurz'. 13. 71 . . . . .	ca. 50	—	—

Subtribus. *Stenopetalinae*.

## STENOPETALUM.

	2n.	n.	Ref. No.
* <i>S. lineare</i> , R. Br. 72 . . . . .	10	—	—
* <i>S. sphaerocarpum</i> , F. M. 72 . . . . .	10	—	—

Subtribus. *Schizopetalinae*.

## SCHIZOPETALUM.

<i>S. Walkeri</i> , Sims. 9a . . . . .	18	—	—
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Tribus. *HELIOPHILEAE*.

## HELIOPHILA.

* <i>H. linearifolia</i> , Bursch. 24a. 25. 73 . . . . .	20	—	—
<i>H. pilosa</i> , Lam. 9a . . . . .	20 I.M.	10	(22)
<i>H. crithmifolia</i> , Willd. 24a . . . . .	20	—	—
<i>H. amplexicaulis</i> , L. 46a . . . . .	20-22	—	—

Tribus. *CREMOLOBEAE*.

## MENONVILLEA.

* <i>M. Gayi</i> , Phil. 71 . . . . .	22	—	—
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## SOURCES OF MATERIAL.

(N.B.—The Name under which a plant was obtained is not necessarily that which has been adopted in this paper.)

*Botanic Gardens.*

- |  |   |
|--|---|
| 1. Belgrade, seed from.                      | 25. Kirstenbosch (South Africa), seed from. |
| 2. Berlin-Dahlem, seed from.                 | 26. Lausanne, seed from.                    |
| 2a. " " , plant in.                          | 27. Leningrad, seed from.                   |
| 3. Bucharest, seed from.                     | 28. Lisbon, seed from.                      |
| 4. Budapest, " "                             | 29. Lithuania, seed from.                   |
| 5. Cambridge, plant in.                      | 30. Lyons, seed from.                       |
| 6. Chelsea Physic Garden, seed from.         | 31. Marseilles, seed from.                  |
| 7. Cluj (Rumania), seed from.                | 32. Montpellier, seed from.                 |
| 8. Coimbra (Portugal), seed from.            | 33. Moscow, seed from.                      |
| 9. Copenhagen (Horto Hauniensis), seed from. | 34. Munich, seed from.                      |
| 9a. Copenhagen (Horto Hauniensis), plant in. | 35. Nancy, seed from.                       |
| 10. Darmstadt, seed from.                    | 36. Nantes, seed from.                      |
| 11. Delft, seed from.                        | 37. Naples, seed from.                      |
| 12. Dublin, seed from.                       | 38. Palermo, seed from.                     |
| 13. Edinburgh, plant in.                     | 39. Paris Museum, seed from.                |
| 14. Ghent, seed from.                        | 40. Pisa, seed from.                        |
| 15. Glasgow, seed from.                      | 41. Royal Hort. Soc. Wisley, seed from.     |
| 16. Gothenburg, seed from.                   | 42. Rome, plant in.                         |
| 17. Göttingen, seed from.                    | 43. Rouen, seed from.                       |
| 18. Halle, seed from.                        | 44. Sophia, " "                             |
| 19. Heidelberg, seed from.                   | 45. Stockholm (Bergianum), seed from.       |
| 20. Helsingfors, seed from.                  | 45a. " " , plant in.                        |
| 21. Hohenheim, seed from.                    | 46. Uppsala, seed from.                     |
| 22. Innsbruck, seed from.                    | 46a. " " , plant in.                        |
| 23. Jena, seed from.                         | 47. Würzburg, seed from.                    |
| 24. Kew, seed from.                          | 48. Zagreb (Yugo-slavia), seed from.        |
| 24a. " " , plant in.                         | 49. Prof. Weiss's private garden.           |
|  | 50. A Manchester commercial grower.         |

## Sources of Wild Material.

- |                                      |                         |
|--------------------------------------|-------------------------|
| 51. Aberystwyth.                     | 64. Rome.               |
| 52. Cambridge.                       | 65. Sicily.             |
| 53. Cornwall.                        | 66. Balkans.            |
| 54. Derbyshire.                      | 67. Teneriffe.          |
| 55. Edinburgh.                       | 68. Madeira.            |
| 56. Ludlow.                          | 69. Persia.             |
| 57. Southport.                       | 70. California.         |
| 58. Caen, France.                    | 71. Chili.              |
| 59. Hyères (Var), France.            | 72. Australia.          |
| 60. Languedoc-Méditerranéen, France. | 73. South Africa.       |
| 61. Starnberger See, near Munich.    | 74. Graz, Austria.      |
| 62. Portugal.                        | 75. San Goar am Rhein.  |
| 63. San Marino, Italy.               | 76. Neusiedel, Austria. |

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June 1931.

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# The New Zealand Species of *Xiphophora* with some Account of the Development of the Oogonium.

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With Plates XVII and XVIII and twenty-eight Figures in the Text.

AMONG a number of algal genera, confined to New Zealand and Australia, we find the genus *Xiphophora*. As this is probably the closest southern representative of *Fucus* this genus is of the utmost interest to us. Till lately there was some doubt as to the number of species to be found in New Zealand, and the essential details of their structure had not been examined. For these reasons I decided to study these points, and I wish to thank Mr. Laing for the untiring assistance and encouragement he gave me in my work.

In the latest reference list of New Zealand Marine Algae, published by R. M. Laing (11, p. 126) we find mentioned two species of *Xiphophora*. These are *Xiphophora chondrophylla* and *X. gladiata*. Both are stated to be found in New Zealand, as well as in Australia. But Agardh (2, p. 7) throws some doubt on the presence of *Fucodium gladiatum* in New Zealand. He shows two varieties of *F. chondrophyllum*, now known as *X. chondrophylla* (R.Br.) Mont, 1892, to be present, but concerning *F. gladiatum*, now *X. gladiata* (Labill) Mont, 1872, he states that among the specimens he has been able to obtain from New Zealand he has seen none which he would have to refer to the true *F. gladiatum*.

The first of the two species to be found was *X. gladiata*. This was described as far back as 1806 by La Billardiere (9, p. 111), who gave it the name *F. gladiatus*. It was not till thirteen years after that *X. chondrophylla* was described by Brown (5). He also placed it in the genus *Fucus*. In 1892 Montague again examined them and changed the generic name from *Fucus* to *Xiphophora*. He also thought fit to change the specific name of *F. gladiatus*, the plant becoming known as *X. Billardieri*. This change was quite uncalled for, La Billardiere having most certainly described the plant. So we find Agardh (1, p. 202) reinstating

the old specific name, besides placing the species in a genus *Fucodium*, which he described to include *Xiphophora*, and other small genera such as *Pycnophycus* and *Physocaulon*. This proved unsatisfactory, and a year later Kuetzing (8, p. 587) reduced the genus *Xiphophora* to *Himantalia*. At last Barton (3, p. 35) restored *Xiphophora* to generic rank, and this is followed by Kjellman in Engler and Prantl (6, p. 281). After such a varied career we can only hope that it will retain its place for a certain number of years.

The original description of *X. gladiata* by La Billardiere is as follows: 'Fucus, fronde compressa, lineari-dichotoma, ramosissima, fusca, intorta, gladiata, Frons pedalis, lineari-dichotoma, ramosissima, fusca, compressa, supra planiuscula, passim intorta, ramulis itidem planiusculis, obtusis longioribus gladiatis, incurvis, ut plurimum furcatis, immersis fructibus foetis. Habitat ad capitis Van Diemen littora.'

Harvey in Hooker's New Zealand Flora (7) gives the following description: '*F. gladiatus*, frond 3-4 in. long, as thick as a swan's quill, terete below, compressed above, dichotomously compound, segments spreading, truncate or two-lobed. Receptacles 6 in. long or more  $1/12$ - $1/6$  in. broad, dichotomously branched, ultimate segments 4 in. long ensiform'.

Through the kindness of Mr. A. H. S. Lucas, I was able to obtain two specimens which had been collected at Hobart in the River Derwent. On examination I found these specimens (Pl. XVII, Fig. 1) to correspond closely with the above descriptions, but to differ considerably from any growing in New Zealand. The oblong wartlike tumours, which La Billardiere supposed to be capsules, I did not find, but other botanists have already attributed them to the result of accidents, since similar growths, occasioned in this way, have been found on other Fuci.

The original description of *X. chondrophylla* by Brown is as follows: 'Fronde between cartilaginous and coriaceous, thick flat linear, nerveless, dichotomous, branches erecto-patent, straight, of equal height, apices emarginate, obtuse. Root, I am at present unacquainted with; frond 8 in. or more long or perhaps extending to a foot, for I have never seen the very base; between flat and compressed, in its lower part from a line and a half to two lines wide, and half a line thick, but then gradually growing more narrow, and more thin as it advances, so that at the apices it is not more than a fourth of either the width or the thickness; at the distance of an inch or two from the root it becomes forked, and is afterwards ten times or more divided in a similar manner, the interval between each dichotomy being very short, the angles of division are acute but somewhat rounded, the margins everywhere entire, sometimes immediately forming the base, irregularly beset with short ligulate shoots of the same nature and substance as the rest of the frond, but in other respects naked; the surface is smooth, and even where the branches are broken, there originate from the

centre of the apices fresh shoots much smaller than the primary ones, which give the frond the same annulated appearance so often observable in *F. lumbricale*'. This proved to be an exact description of some plants forwarded to me by Miss L. Cranwell, from the Bay of Islands and the districts around Auckland (Pl. XVII, Fig. 2). I therefore consider these to be the same as the typical *F. chondrophyllus* discovered by Brown.

The chief points of difference displayed by the two species mentioned above are as follows:

(1) *X. gladiata* has a much more open form of growth than *X. chondrophylla*.

(2) The very rounded axils of the former are quite distinct from those of the latter which, though rounded, are very much more acute.

(3) The dichotomy of *X. gladiata* is somewhat regular and infrequent in contrast to the crowded dichotomy displayed by *X. chondrophylla*.

(4) The bases of the two species are quite distinct, that of *X. gladiata*, being narrow, round, and quill-shaped, while *X. chondrophylla* has a base that is somewhat wider and always remains compressed.

(5) The receptacles of the former are long and swordlike, while in the latter they are quite short and are given off in tufts.

It is this last point which has caused such controversy as to the presence of *X. gladiata* in New Zealand, since a number of specimens of *Xiphophora* are found which possess these long swordlike receptacles so distinct from those of the *F. chondrophyllus* described by Brown (Pl. XVIII, Fig. 6). Agardh (2, p. 7) was the first to examine this point, and he came to the conclusion that two varieties of *X. chondrophylla* were to be found in New Zealand. These he called *Fucodium chondrophyllum* var. *α minus* and *F. chondrophyllum* var. *β maximum*, the former variety agreeing entirely with Brown's description, while the latter includes specimens which possess long swordlike receptacles, but which resemble *X. chondrophylla* in most other respects. Agardh's (2) definition of the two varieties is as follows:

*α minus* scarcely a foot long, the narrow receptacles showing on each leaf almost a single longitudinal series of conceptacles.

*β maximum* a foot long or more, the small inner receptacles showing conceptacles arranged in several series on each leaf.

All specimens which I was able to obtain from New Zealand agreed with one or other of these two descriptions.

The first of these two varieties, *X. chondrophylla* var. *minus*, is found in the northern parts of New Zealand, and does not extend further south than the Auckland District. The following is a list of the localities in which it has been found: Whangapoua, Great Barrier Island, Parenga, Tauranga, Rangitoto Reef, Malonys Reef, Bay of Islands, Hokianga, Motuihis

Monganui, Coromandel, and Poor Knights Island. Berggren in Agardh (loc. cit.), states it to have been found in Lyall Bay, Wellington, but I have never seen a specimen from there, and think it most unlikely that a single plant should be found so far south. I myself have spent a considerable time looking for specimens at Lyall Bay, and have only been able to find the larger variety of *Xiphophora*. All the specimens I was able to obtain from the above districts resembled Brown's diagram and description very closely. The one exception was the plant from Poor Knights Island (Pl. XVII, Fig. 3). This, in the method of its branching and in long swordlike receptacles, resembles var. *maxima* very closely, but since these receptacles are narrow and possess but a single longitudinal series of conceptacles it must be classed as var. *minus*. This difference in form shown by the plant I take to be an epharmonic variation, due to the plant growing in a tidal estuary where the surge of the sea reaches its extreme force. The other plants, though growing on rocks in a tidal zone, would not be subjected to quite the same strain and would not need to assume the form of the Poor Knights plant. It is interesting to note that var. *maxima*, the specimens of which typically possess long swordlike receptacles, is always found growing in a considerable surge, and never where the sea is comparatively calm.

*X. chondrophylla* var. *maxima* is widely distributed in New Zealand, its limit being the far north where var. *minus* holds sway. It is found in Wellington at Lyall Bay, Island Bay, and Breaker Bay. Farther south in Banks Peninsula it is found at Le Bons Bay, Wainui, and Oahoa Bay, while in Otago it may be found at St. Clair, Brighton, the Bluff, Harrington Point, and Papanui Bay. Among the outlying islands where it can be found are Auckland Islands, Campbell Island, the Chatham Islands, and the Antipodes.

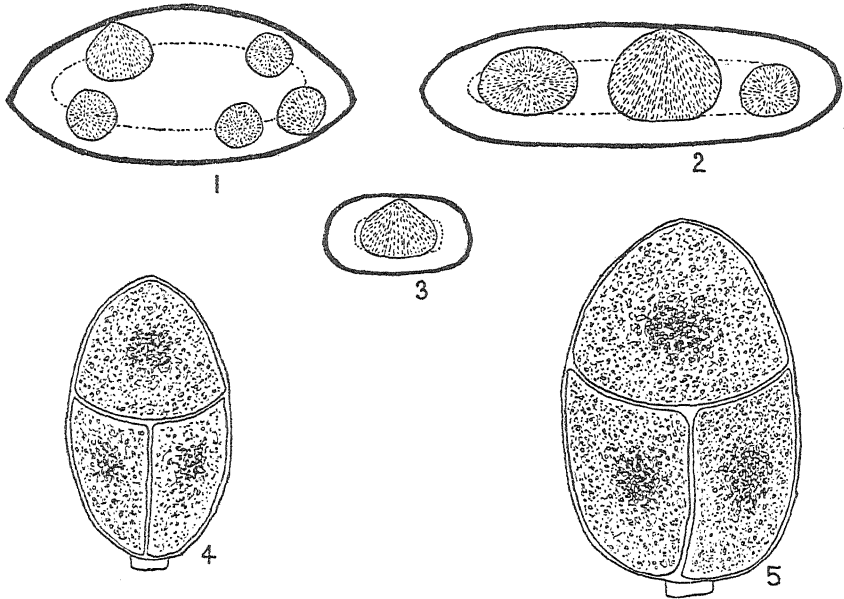
Specimens from these various places all agree with Agardh's definition, the linear receptacles showing conceptacles arranged in several series in each leaf. All except the very young forms were also found to be a foot or more long. Two other points in which they differ from var. *minus* are also worthy of note. One is the fact that the receptacles are long and swordlike, resembling those of *X. gladiata*, and the other is that their base is usually much more fleshy and most distinctly flattened (Pl. XVIII, Fig. 5). On the whole, specimens of this group were not as uniform in their outward appearance as var. *minus*. These variations, however, I discovered to be chiefly due to the differences in age. I was able to obtain a complete series representing the different forms at Lyall Bay. All of these were gathered at the same place, and therefore were subjected to the same ecological conditions. The youngest of these (Pl. XVII, Fig. 4) was not more than 15 cm. long, and in outward appearance was very similar to young plants of var. *minus*. Its numerous branches, nearly all of which

were still actively dividing, all terminated at approximately an equal height from the base of the plant. It, however, already has a few receptacles which, with their conceptacles arranged in two rows, showed it to belong to var. *maxima*. The oldest specimen of the series gave a more typical appearance of var. *maxima*. It was fully 50 cm. long and its base was flat and fleshy, being 3 mm. in thickness and 20 mm. wide. The vegetative branches were numerous, and mostly terminated in long tapering points instead of the comparatively abrupt endings of the branches of the younger plant. The receptacles were long and swordlike, and likewise terminated in long slender points: towards the base they were, however, found to be as much as 6 mm. across. Dichotomy did not appear to be so frequent as in the younger plant. Between these two extremes I found a number of plants which formed a gradual chain from one to the other, so that the variations in size and shape appear to be primarily due to difference in age, the younger plants resembling var. *minus* to some extent, while the older ones are quite distinct. In support of this view I possess a young plant which from its appearance might be either var. *minus* or var. *maxima* (Pl. XVIII, Fig. 7). It is but 4 cm. high, and with its numerous short branches is very like var. *minus*. It bears no receptacles as yet, but as it was found in Otago in the midst of larger specimens of var. *maxima* it is reasonable to assume it to be a very young plant of the latter. The variations which are brought about by ecological conditions are only slight. As in var. *minus*, the plants which are subjected to a greater tidal force are more slender and tapering than those that live in comparatively calm waters.

The internal structure of the various species give further support to the view that the specimens found in New Zealand are quite distinct from *X. gladiata* in Australia. On examining the sections of the receptacles, as seen in the transverse section of the thallus of the various species, certain differences can easily be noted (Text-figs. 1, 2, 3). In *X. gladiata* the conceptacles are comparatively small and are scattered round the margin of a slightly thickened thallus (Text-fig. 1). In *X. chondrophylla*, on the other hand, the thallus is distinctly flattened and the conceptacles are arranged in regular rows (Text-figs. 2 and 3), while the conceptacles are approximately twice the size of those of *X. gladiata*. The difference between the two varieties of *X. chondrophylla* may also be seen in the number of rows of conceptacles. In var. *minus* a single row is found in the centre of the receptacle, while in var. *maxima* two to three rows may be found. The conceptacles are, however, of the same size. Specimens of *X. chondrophylla* from Australia proved to be similar.

Another point of difference is that the oogonia of *X. gladiata* are only half the size of those found in both varieties of *X. chondrophylla* (Text-figs. 4 and 5). This again emphasizes the fact that the two species

*X. gladiata* and *X. chondrophylla*, are different, while the two types of *Xiphophora* found in New Zealand, though so different in external appearances, are fundamentally too similar to be separated into two distinct species, and can only be looked upon as varieties of the same species.

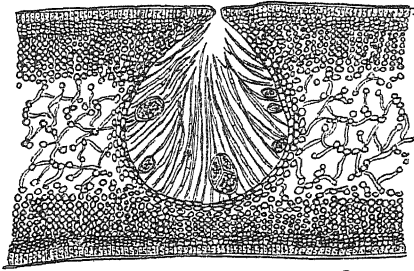


TEXT-FIGS. 1-5. 1. Longitudinal section of receptacles of *Xiphophora gladiata*.  $\times 4$ . 2. Longitudinal section of receptacles of *X. chondrophylla* var. *maxima*.  $\times 4$ . 3. Longitudinal section of receptacle of *X. chondrophylla* var. *minus*.  $\times 4$ . 4. Oogonium of *X. gladiata*.  $\times 170$ . 5. Oogonium of *X. chondrophylla*.  $\times 170$ .

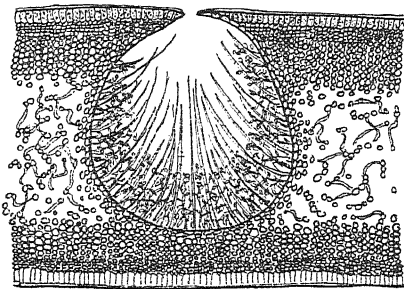
The only description of the internal structure of the *Xiphophoras* which has so far been published is that of Barton (3, p. 36). She gives a short account of the structure of specimens from Lyall Bay which she calls *X. gladiata*, but which probably were *X. chondrophylla* var. *maxima*. As she was only able to work on dried material, most likely not in the best state of preservation, I found several inaccuracies no doubt due to this handicap. Like her, I found the thallus to be composed of three tissues of cells (Text-fig. 8). These are as follows: (1) the cortex, consisting of a single row of long narrow radiating cells; (2) the parenchyma, below the cortex, consisting of rounded cells closely packed; and (3) a central strand of hyphae-like cells, which ramify loosely and present various shapes according to the angle at which they are cut, sometimes appearing round or oval, at other times long and filamentous. I was not able to discover any pits in the longitudinal walls of these septate cells. Both species proved to be monoecious and the conceptacles are pear-shaped (Text-figs. 6 and 7). The oogonia divide tetrahedrally to form ova (Text-fig. 9) and possess a pedicel cell, which Barton did not believe to be present.



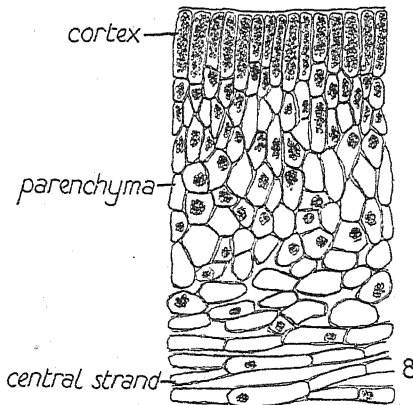
I also differed from her in finding the antheridial branches to be septate (Text-fig. 10) and considerably thicker than they are shown to be in her



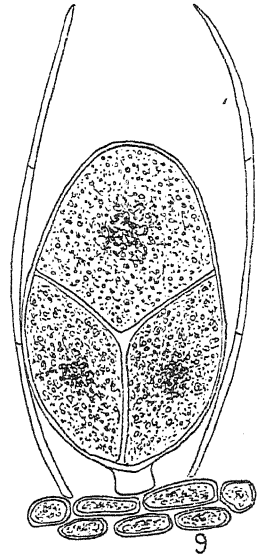
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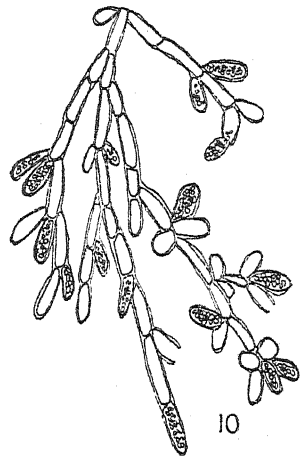
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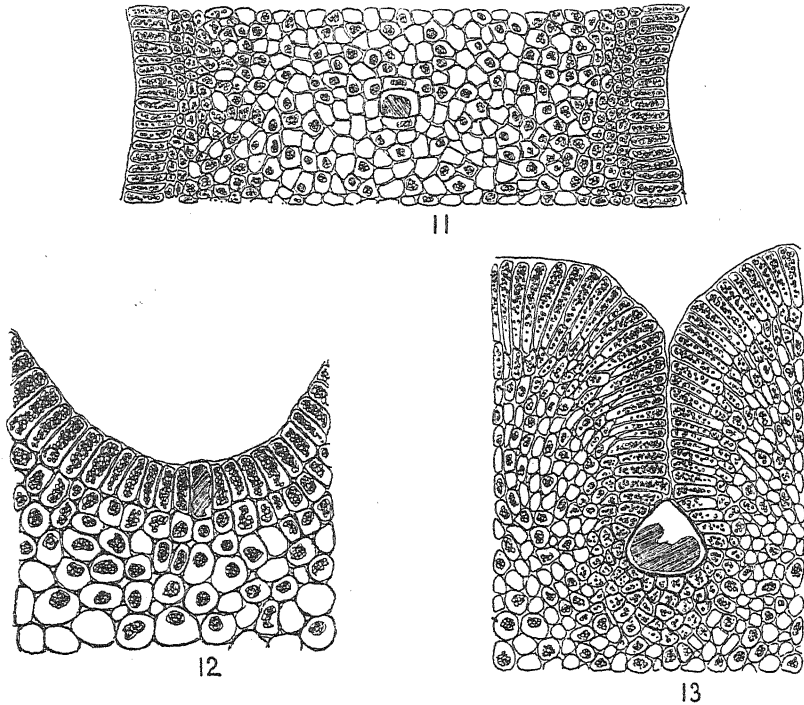


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TEXT-FIGS. 6-10. *X. chondrophylla*. 6. Longitudinal section of receptacle showing female conceptacle.  $\times 20$ . 7. Longitudinal section of receptacle showing male conceptacle.  $\times 20$ . 8. Transverse section of thallus showing different layers of cells.  $\times 170$ . 9. Oogonium.  $\times 170$ . 10. Antheridial branch.  $\times 170$ .

diagram. On the whole the structure of the thallus appears to be very similar to that of *Fucus*. This similarity is further emphasized by an examination of the apical cell and the development of the conceptacles.

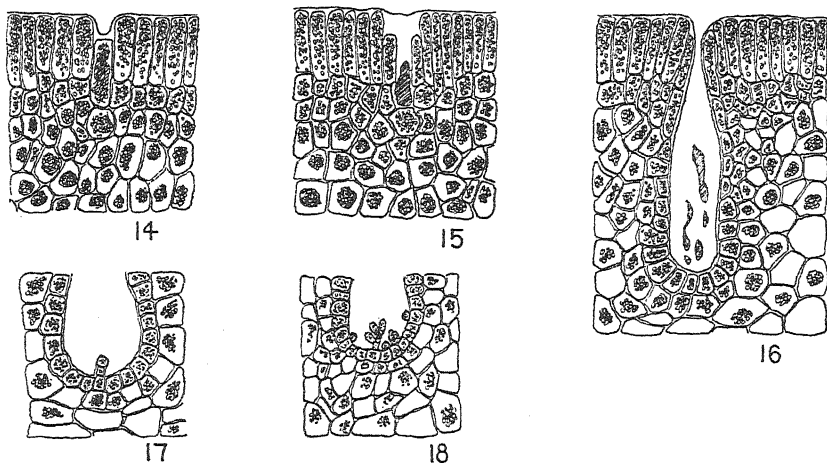
In dividing the Fucales into several groups, Oltmann (13, p. 189) states that some importance is attached to the shape of the dividing cell. This in the Fuco-ascophylleae, to which *Fucus* and *Xiphophora* belong, is



TEXT-FIGS. 11-13. *X. chondrophylla*. 11. Transverse section across tip of thallus on a plane just below the bottom of the depression.  $\times 170$ . 12. Longitudinal section of tip of thallus parallel to the broad surface of the frond.  $\times 170$ . 13. Longitudinal section of thallus in a plane at right angles to the terminal depression.  $\times 170$ .

found to be a peculiar four-sided dividing cell. A detailed account of the apical cell of *Fucus* is given by Woodworth (14), with which description I found the dividing cell of *Xiphophora* to agree closely. I cut three sections at right angles to each other, the first longitudinally in a plane at right angles to the terminal depression (Text-fig. 13), the second, at right angles to the last, that is, parallel to the broad surface of the frond (Text-fig. 12), and lastly, a section across the tip on a plane just below the bottom of the depression (Text-fig. 11). In all these sections one cell appears larger than the rest and stains more deeply. Smaller cells can be seen dividing from it in different directions. Just below the large cell are small irregular shaped cells from which the hyphae of the stem gradually take their origin. The cells on either side of the central cell divide again in three directions at right angles to each other, the upper portion of the cells thus formed becoming epidermal cells, while the lower ones form the

cortex. From these three different sections we can reconstruct the apical cell as being a four-sided wedge-shaped cell, with convex sides and the largest diameter at right angles to the broad surface of the frond. The smaller and upper end is rounded and the base truncated.

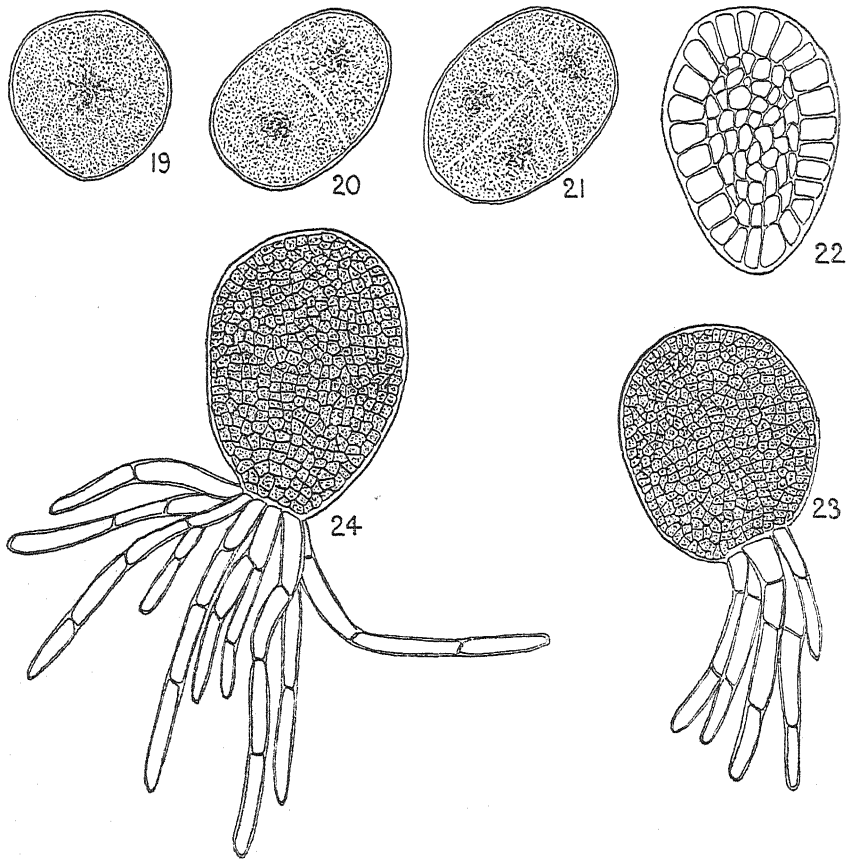


TEXT-FIGS. 14-18. *X. chondrophylla*. 14. Transverse section of thallus showing initial and basal cells of young conceptacle.  $\times 170$ . 15. Transverse section of thallus showing collapse of wall of initial cell of young conceptacle.  $\times 170$ . 16. Transverse section of thallus showing formation of thin-walled cells lining the cavity of the conceptacle.  $\times 170$ . 17. Transverse section of thallus showing surface cells of conceptacle projecting into the cavity.  $\times 170$ . 18. Transverse section of thallus showing initial formation of antheridial hairs in the conceptacle.  $\times 170$ .

The development of the conceptacles of *Xiphophora* was likewise found to be similar to that of *Fucus*. The youngest stages are situated close to the apex of the branches which still retain active apical growth. As in *Fucus*, there was found to be present an initial cell, with a basal cell immediately beneath it (Text-fig. 14). Later the cell-walls of the initial cell collapse and the contents form a swollen mass which fills the cavity (Text-fig. 15). The basal cell increases in size, and divides to form a layer of thin-walled closely packed cells which line the cavity (Text-fig. 16). The cavity is at first long and narrow, but as it increases in size it also widens, ultimately becoming pear-shaped. With this gradual increase in size, surface cells of the cavity can be seen to project into it, forming papillae (Text-fig. 17). As the conceptacles develop, these papillae gradually divide, forming the various sexual organs and hairs which accompany them (Text-fig. 18). So that in all the fundamental details the development of the conceptacles of *Xiphophora* agrees with that of *Fucus*.

The sections for studying the development of the conceptacle and the shape of the apical cell were prepared by fixing fresh pieces of *Xiphophora* in Bouin's solution and then imbedding in the usual way. The slides were stained with eosin, in which they were immersed for a few minutes.

To study the development of the oogonium I liberated some oogonia and antherozoids into a Petri dish of salt-water. I obtained these from plants growing at Lyall Bay. These plants bore receptacles which were

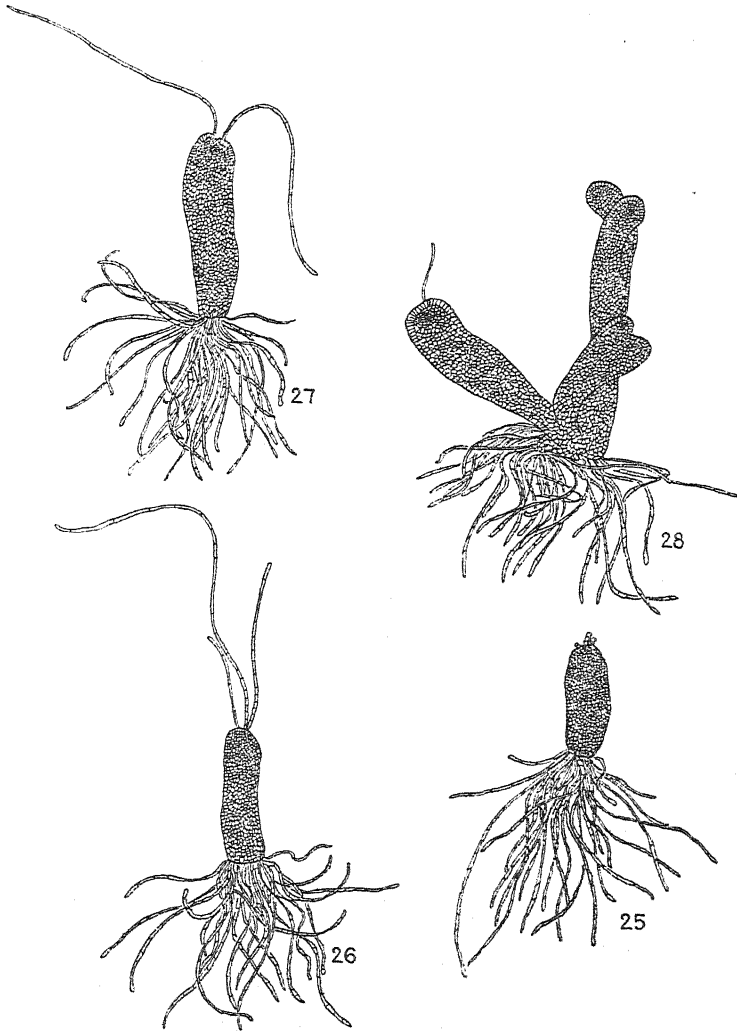


TEXT-FIGS. 19-24. *X. chondrophylla*. 19. Ovum before germination.  $\times 170$ . 20. First stage of division of fertilized ovum.  $\times 170$ . 21. Second stage of division of fertilized ovum.  $\times 170$ . 22. Longitudinal section of young plant in the earlier stages of division.  $\times 170$ . 23. Young plant showing formation of rhizoids. 24. Young plant with rhizoids further developed.  $\times 170$ .

covered with clusters of orange antherozoids in some cases, and with olive-green oogonia in others. I was thus able to obtain a large number of both in a ripe condition. Germination took place within a week after fertilization. The first line of cleavage was transverse, the second vertical (Text-figs. 20 and 21), after which divisions took place in quick succession, the embryo dividing up into a number of small cells. A longitudinal section at this stage shows the cells of the young plant to be oblong in shape and fairly regular. The outer ones being slightly longer and radiating towards the

centre, with their long edges pressed close together, while the inner ones are slightly smaller and are not packed so tightly (Text-fig. 22).

The young plant increases in size, and soon at one end hairlike pro-



TEXT-FIGS. 25-28. *X. chondrophylla*. 25. Young plant with papillae-like protrusions forming at upper end.  $\times 30$ . 26. Young plant with hairs fully developed.  $\times 30$ . 27. Young plant becoming flattened and forming a groove at the tip.  $\times 30$ . 28. Young plant with branches beginning to divide dichotomously.  $\times 30$ .

cesses are seen to form (Text-fig. 23). These are the rhizoids, which increase rapidly in size and number till they form a large tuft, even bigger than the rest of the plant (Text-fig. 24). After five or six weeks small

hairs form at the opposite end. These first appear as small papillae-like protrusions (Text-fig. 25). However, these soon divide to form a long row of regular cells. At first these cells have their greatest diameter in a horizontal direction, but they soon increase in length till their greatest diameter is in the vertical direction. The hairs therefore grow in length, both by cell-division and by the increase in length of the cells composing them. They finally become long undulating hairs, about twice as long as any of the rhizoids (Text-fig. 26). No more than four or five hairs ever seem to be formed.

After three or four months further changes can be noticed in the plant. An apical cell can now be seen to form, while the whole plant becomes flattened and a groove forms at the tip (Text-fig. 27). Gradually this groove deepens, the hairs disappear, and the plant begins to divide dichotomously. In this way the plant becomes gradually more and more similar to the adult form in appearance (Text-fig. 28).

On comparing this development of the fertilized egg of *Xiphophora* with that of *Fucus*, described by Oltmann (13), we find a certain similarity. The rhizoids of the former, however, are unlike those of the latter in being much more slender and hairlike, and in not arising from thick papilla-like protrusions, as are indicated in the diagrams of young *Fucus* plants. The hairs also differ somewhat, since they never occur in such numbers as in *Fucus* and vary in length, some being considerably longer than any found in the latter. The fundamental changes which the fertilized egg goes through in its germination and development, however, seem to be identical in *Xiphophora* and *Fucus*.

From the above account the assumption that the genus *Xiphophora* is probably the closest southern representative of *Fucus* appears to be correct. It will be interesting to see what other New Zealand algae on examination show such a resemblance to their northern relations both in structure and development.

#### SUMMARY.

1. Of the two *Xiphophora* species—*X. gladiata* and *X. chondrophylla*—only the latter is found to be present in New Zealand.
  2. The distinguishing characters of each species are given.
  3. The characters and distribution of the two varieties of *X. chondrophylla* in New Zealand are discussed.
  4. An account is given of the internal structure, the dividing cell, and the development of the conceptacle.
  5. The development of the oogonium is described.
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EXPLANATION OF PLATES XVII AND XVIII.

Illustrating Miss Heine's paper on The New Zealand Species of *Xiphophora* with some Account of the Development of the Oogonium.

PLATE XVII.

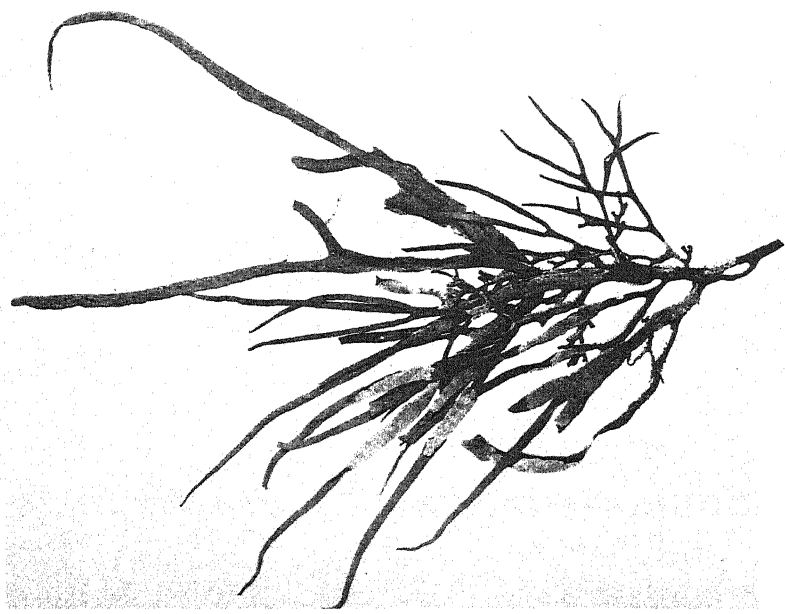
- Fig. 1. *X. gladiata*. Port Darwin, Tasmania.  $\times \frac{1}{2}$ .  
 Fig. 2. *X. chondrophylla* var. *minus*. Bay of Islands, N.Z.  $\times \frac{2}{3}$ .  
 Fig. 3. *X. chondrophylla* var. *minus*. Poor knights Island.  $\times \frac{1}{2}$ .  
 Fig. 4. *X. chondrophylla* var. *maxima*. Young plant, Lyall Bay, N.Z.  $\times \frac{2}{3}$ .

PLATE XVIII.

- Fig. 5. *X. chondrophylla* var. *maxima*. Mature plant, Lyall Bay, N.Z.  $\times \frac{1}{3}$ .  
 Fig. 6. *X. chondrophylla* var. *maxima*. Showing long swordlike receptacles.  $\times \frac{1}{3}$ .  
 Fig. 7. *X. chondrophylla* var. *maxima*. Very young plant, Otago, N.Z.  $\times 1$ .



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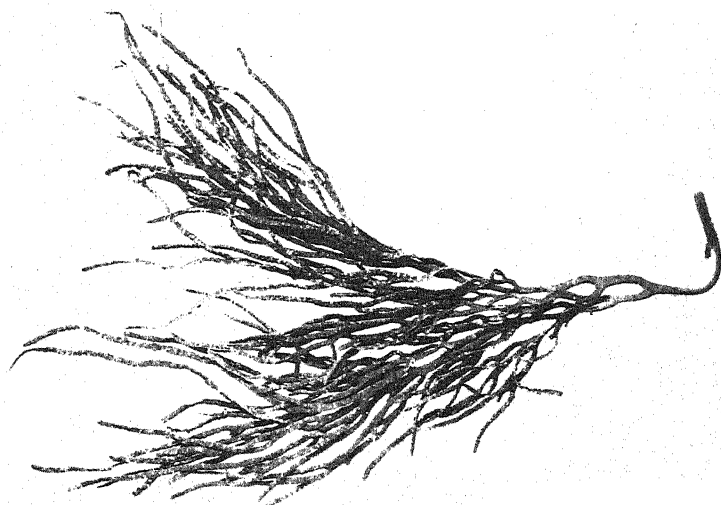


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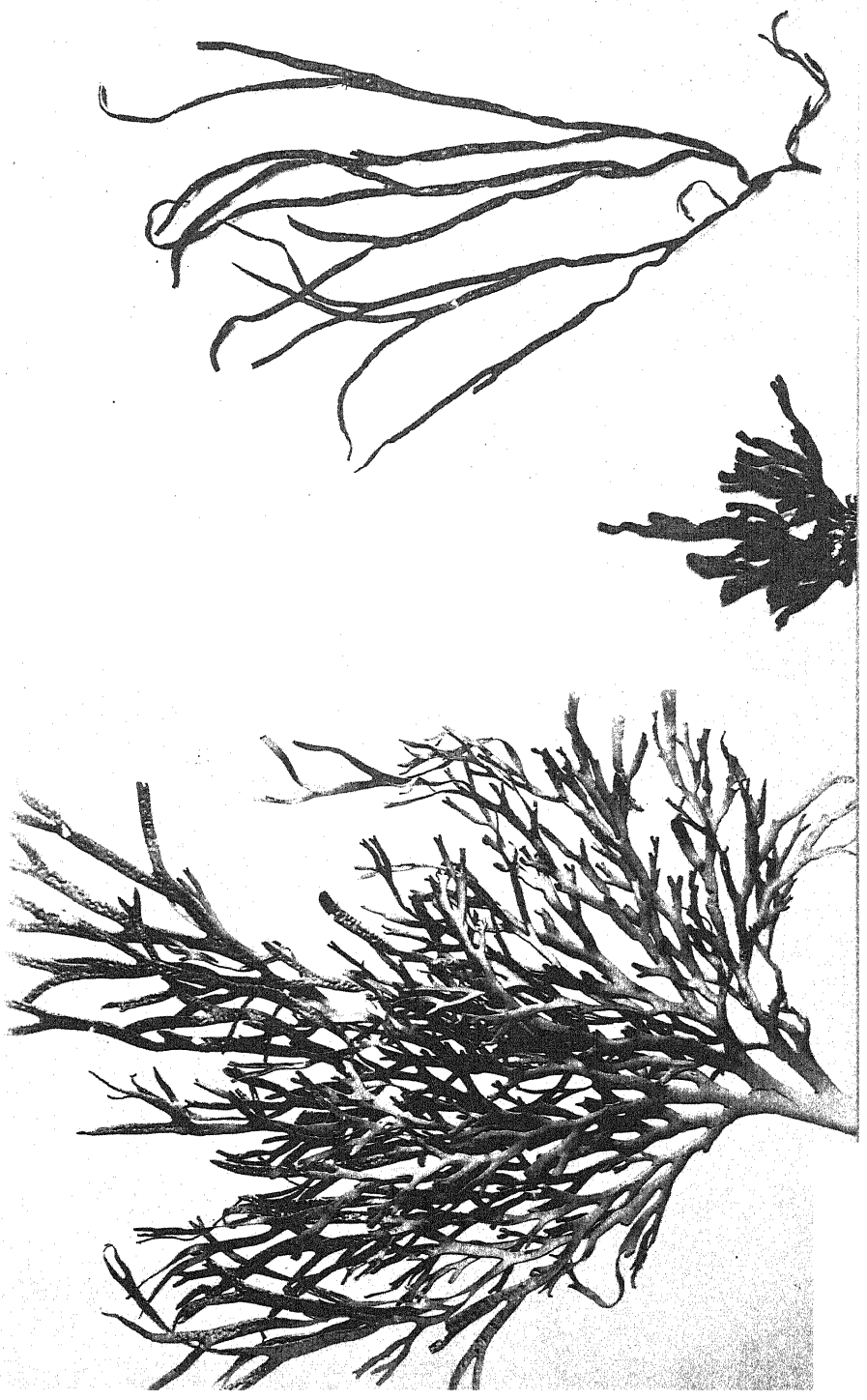


4



3







# The Absorption of the Solute from Aqueous Solutions by the Grain of Wheat.

BY

R. BROWN.

*(Botanical Department, Seale-Hayne Agricultural College, Newton Abbot.)*

With three Figures in the Text.

KNOWLEDGE of the conditions regulating the absorption of various substances by the seed is essential to the effective prosecution of researches on the chemical treatment of seeds. The present series of investigations were undertaken when it was found that the data available on the absorption of chemical compounds by the seed were inadequate for certain researches in progress on the chemical stimulation of rye-grass seeds. For the researches described in the sequel wheat was chosen as the experimental material, in order to eliminate complications which might be introduced by the pales.

It has been generally assumed that the chief limiting factor to the absorption of any substance from solution is the semi-permeable membranes of the seed-coat. The first detailed investigation of the properties of the semi-permeable membranes of the seed-coat was made by A. J. Brown (3, 4). He says 'there seems to be no doubt that the semi-permeable property of the barley grain centres in the spermoderm'. He was unable, however, to determine the exact nature of the semi-permeable layer. Later Reichard (11) attempted to identify the semi-permeable layer with certain deposits of tannin that he found in the seed-coat of the barley grain. Shull (13) demonstrated the presence of semi-permeable membranes in a large variety of seeds, and in the seeds of one particular genus, *Xanthium*, he localized the semi-permeable layer. In the case of *Xanthium* he attributed semi-permeable properties to the two innermost layers of the testa. 'Both layers,' he says, 'possess osmotic properties, the inner layer in a higher degree than the middle one.'

Collins (7) investigated the semi-permeable system of the barley grain, and came to the conclusion that semi-permeable properties were present only in the mucilaginous chalazal cells opposite the micropyle. He

suggested that absorption was only possible along this localized tract because the rest of the seed was covered by membranes which he identified as cuticular and, therefore, presumably impermeable to water and all solutes. Since the publication of Collins' work several investigators, whose work will be discussed in a later section, have been unable to confirm Collins' findings of the impermeability of the seed-coat to water and solutes. Netolitzky (9) has recently examined the nature of the semi-permeable layers, and he identifies them with the layers of cuticle that had been previously noted by Collins.

The present writer has re-examined the problem of the nature of the semi-permeable layers, and has reached conclusions widely divergent from those discussed above. The problem was investigated by soaking seeds in various solutions and later determining the depth to which the solute had penetrated by microscopical examination. The seeds were soaked in various dyes, in sodium chloride followed by silver nitrate, and in silver nitrate alone. Sections from seeds soaked in the latter solution were exposed to light, and the distribution of the black deposit could be followed with ease. In the earlier stages of the experiment seeds were examined at intervals of two hours, later the period in the same experiment was extended to six hours. It was found that the time intervals noted between each stage of absorption as detailed below were approximately the same whatever the solute. For a long time the solute penetrated only as far as the so-called 'cuticular membrane' (Collins and Netolitzky) attached to the outer surface of the single layer of the testa. The solute seems to accumulate and is deposited in a sharp line on the outer surface of this cuticle. Later the solute appeared in the single layer of the testa. At 75 hours after immersion the solute penetrated a thinner cuticular membrane attached to the inner surface of the single layer of the testa. The solute is not deposited in a sharp line on the outer surface of this second cuticular membrane, but seems to accumulate in the walls of the testa cells. The deposition of the solute on the outer surface of the cuticle on the outer surface of the testa suggests that the former has semi-permeable properties. The inner cuticular membrane being apparently of the same composition might be expected to have the same properties, and therefore semi-permeability can be inferred for this membrane too. The accumulation of the solute in the testa indicates that it is being withheld from the inner cuticle. The walls of the testa have, as we shall show later, colloidal properties, in which case the adsorption of the solute in this layer might be expected. The conclusion would seem to be that we are dealing with a semi-permeable system, consisting of two cuticular membranes enclosing a layer that is structurally and chemically different.

The evidence for regarding the membranes on the inner and outer surfaces of the testa as different from normal cuticle has been discussed

elsewhere (6). Briefly, however, the microchemical reactions supporting this contention are

1. The membranes turn red after application of KOH and Alkannet more readily than does the cuticle of the foliage leaf,
2. They turn a brownish colour with osmic acid,
3. They turn faintly red with Sudan III solution alone.

These results seem to indicate that true fat enters into the composition of these membranes to a greater extent than it does in the cases of cuticle such as that on the outer surface of the epidermis of the foliage leaf. The permeability of the cuticle-like membranes of the seed to water and solutes cannot, therefore, be compared with that of the cuticle of the leaf. The greater fat content of the former might be expected to result in a greater permeability. The single layer of cells between the two cuticle-like membranes reacted towards water in a manner suggestive of mucilage—the cells swell in water and contract when treated later with alcohol. The layer gave positive reactions with cellulose tests, and also gave positive reactions when tested microchemically for mucilage.

The cuticle-like membranes are not of uniform thickness over the whole seed. They are very much thinner at the base than they are over the rest of the seed. Different rates of absorption over the same seed corresponding to these differences in thickness of the semi-permeable membranes might therefore be expected. Variations in the rate of absorption over different parts of the seed have been noted by a number of earlier investigators. Schroeder (12) noted an earlier absorption at the base later spreading to the apical portions of the seed. Such he attributed to a gradient of permeability in the seed-coat. Collins, in a paper already noted, describes a similar sequence, but he explained this by assuming an upward diffusion of the solute in the sub-aleuronic cells of the endosperm. Beeskow (1) and Braun (2), however, have shown that there is no sub-aleuronic diffusion of iodine. Braun adduced this as further evidence of Schroeder's theory of a gradient of permeability. This theory has also lately been supported by Shull (14). The present investigator in an earlier paper on the absorption of water by the gramineous seed could find no evidence for assuming a gradient of permeability, but adduced evidence to show that the absorption of water was regulated by the degree of stretching of the seed-coat. Water, which is first absorbed at the micropyle, diffuses into a drier layer of the endosperm, the seed at this higher level swells and stretches the seed-coat, with consequent increase in the permeability of the latter. The water that now enters at this higher level enables a further upward diffusion, and in this way the area of absorption spreads towards the apex, the increased permeability consequent upon stretching being due to an increase in the size of the inter-molecular spaces.

The paths followed by the solute during absorption have been re-examined, and the results achieved amplify those of earlier workers. The method used was that of soaking the seeds in various solutions, removing them from these solutions at regular intervals, and examining longitudinal sections made at a plane at right angles to that of the furrow. The solutions were iodine, trichloroacetic acid, phenol,  $\text{AgNO}_3$ , various dyes, and  $\text{NaCl}$  followed by  $\text{AgNO}_3$ .

The general sequence fell into one of two classes:

1. Where the membranes of the seed are freely permeable to the solute, the absorption of the latter follows closely that of water. The absorption of iodine, which is an example of this type of absorption, has been described very fully by several authors (2, 6, 7, 12). The iodine stained area of the endosperm is at first confined to the base of the seed, but as the area of water absorption spreads upwards, so also does the area of iodine absorption. It would seem that the iodine can only enter when the seed coat has been stretched by the internal swelling of the seed, consequent upon the absorption of water. At no stage of absorption does there seem to be any accumulation of the solute in any part of the seed coat.

2. When the membranes of the seed are only slightly permeable to the solute, the sequence in absorption differs from the above. The absorption of  $\text{AgNO}_3$  is an example of this second type. The earliest absorption takes place through the micropyle at the base. Over the remainder of the seed during the first 40 hours the salt penetrates only as far as the cuticle on the outer surface of the testa. Later the solute diffuses slowly into the testa, this process taking place almost simultaneously over the whole seed. From 40 to about 60 hours the diffusion continues slowly across the testa, and small amounts of the salt reach the endosperm. After 60 hours the salt reaches the endosperm very much more rapidly. It seems that at about this time there is a partial breakdown in the semi-permeable system.

The nature of the absorption-time curve is suggestive in connexion with the differences noted above.

The quantitative determination of absorption was effected by a method similar to that used by A. J. Brown. Weighed samples of 100 seeds are placed in a test-tube to which 4 c.c. of the solution are added. Successive determinations are each made on a different sample of seed, so that the figures do not represent absorption by a single sample of seed. The seeds and the supernatant solution are poured into a separate vessel, any solution left adhering to the walls of the test-tube is washed out with at least three changes of distilled water. The solution, together with the washings, are then decanted off the seeds into a second vessel. The seeds themselves are washed with at least four changes of water, the washings being added



each time to the original solution and the washings from the test-tube. Wolfe (15) and Kotowski (8) have lately pointed out the necessity of very thorough washing of the seed after removal from the solution. The amount of solute not absorbed by the seed is now determined by the ordinary method of volumetric analysis. The method is by no means ideal; it is open to the objection that it is impossible to obtain figures for comparative purposes from different samples of seed each having the same surface area. Attempts were made to establish some relationship between weight and absorption. The results, however, were very irregular. The relationship between the two variables was certainly not a linear one. In the present investigation samples were selected as uniform as possible, and it has been assumed that the surface areas involved are the same in all cases.

The absorption of iodine by the seed is represented in graphical form in Fig. 1. It will be noticed that the curve is regular and exponential and of the form represented by the equation

$$y = ae^{dx}$$

when  $y$  = amount absorbed,  
 $x$  = time,  
and  $a$  and  $d$  are constants.

Brown and Tincker (5) found that the absorption of certain readily absorbed organic compounds followed the same law. It would, therefore, seem that whenever the absorption is not retarded by the seed-coat then the curve representing absorption is an exponential one.

The slow absorption rate of electrolytes is therefore probably due to the electrical charges carried by their constituent ions.

The absorption of NaCl and HCl is represented in graphical form in Figs. 2 and 3. The absorption-time curve in these cases is more complex. The lower parts of the curves are parabolic, and they are each presented by separate equations of the type  $y = dx^2$ . The sudden deflexion in rate that occurs at about 48 hours correspond in time with the breakdown of the semi-permeable system noted earlier, and the greater rate to which the deflexion is due is probably a result of this change in permeability. A further indication that this change in rate is due to the conditions of the seed-coat arises from the fact that when the seed is cracked and the influence of the seed-coat is eliminated, the absorption is regular. It would seem probable that the phenomenon is an electrical one; it is only found during the absorption of electrolytes. The absorption of non-electrolytes proceeds more regularly. The adsorption of the solute by the mucilaginous testa does not seem to be affected by the degree of dissociation of the solute. The accumulation of non-dissociated dyes was noted in this layer. It would, therefore, seem that the electrical forces responsible for this retardation are located in the cuticle-like membranes. If it is assumed that the

membrane is traversed by a series of channels, and that the walls of these channels carry a certain electrical charge, then free diffusion across the membrane by an electrolyte will not take place until the charge has been

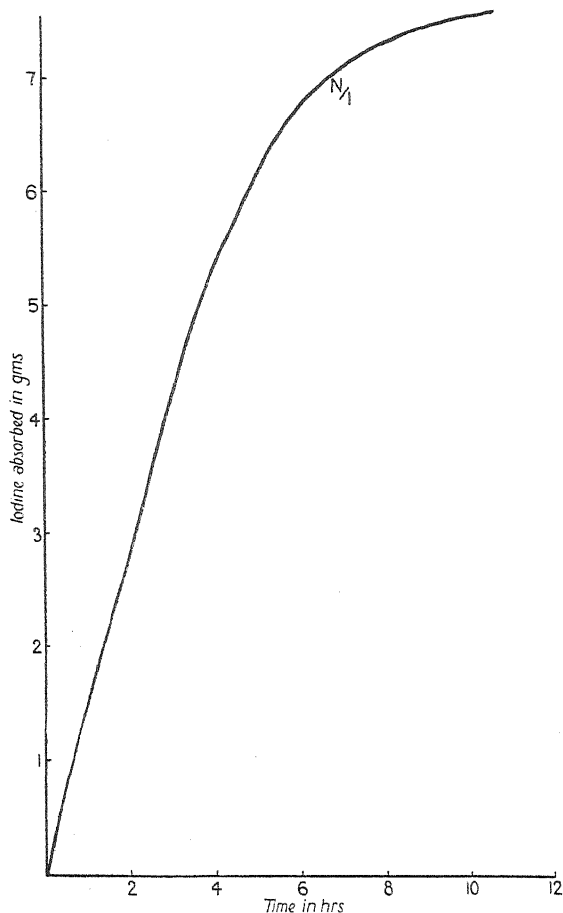


FIG. 1. Rate of absorption of iodine from a normal solution in grams per 100 grams of seed.

neutralized by the adsorption of oppositely charged ions. The point at which increase in permeability takes place would, on this view, correspond to the point at which electrical adsorption by the membrane was complete. The electrical charge in the walls of the channels would clearly not affect a compound that is not electrolytically dissociated. The time from the beginning of absorption at which the acceleration in the rate sets in tends to increase as the concentration of the solution falls. Such might be expected in view of the fact that the same quantities must be adsorbed in order to neutralize the same charge. Nevertheless, the quantity absorbed

at the point at which the charge occurs is not the same in all cases. The explanation probably lies in the fact that the figures for total absorption include absorption at the base of the seed, where the process is not retarded

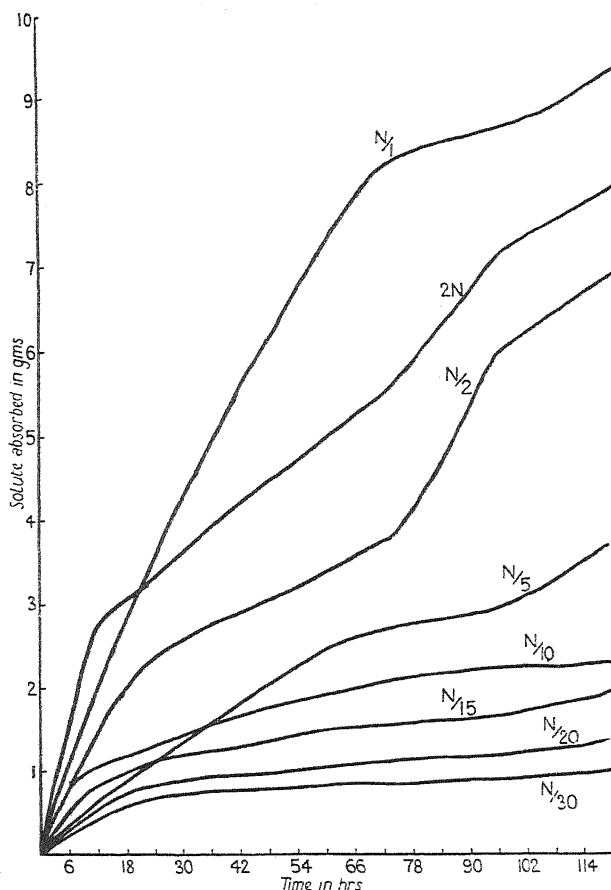


FIG. 2. Rate of absorption of hydrochloric acid from aqueous solutions of varying concentration in grams per 1,000 grams of seed.

by the presence of a thick cuticular membrane. The accumulation of the solute on the outer surface of the outer cuticle suggests that whatever the concentration the amount of solute that reaches the testa is the same in all cases.

Shull (14) has recently shown that the molecular non-dissociated form of any of the halogens is more readily absorbed by the seed than is the ionic form of the same substances. A few preliminary determinations have been made with certain chlorides, nitrates, and hydroxides to test the effect of the degree of dissociation of the salt on the rate of absorption.

It has been found that the rates of absorption are inversely in the order of the degrees of dissociation.

It would, therefore, seem from the above that the rate at which the solute reaches the endosperm is regulated by a process of adsorption that

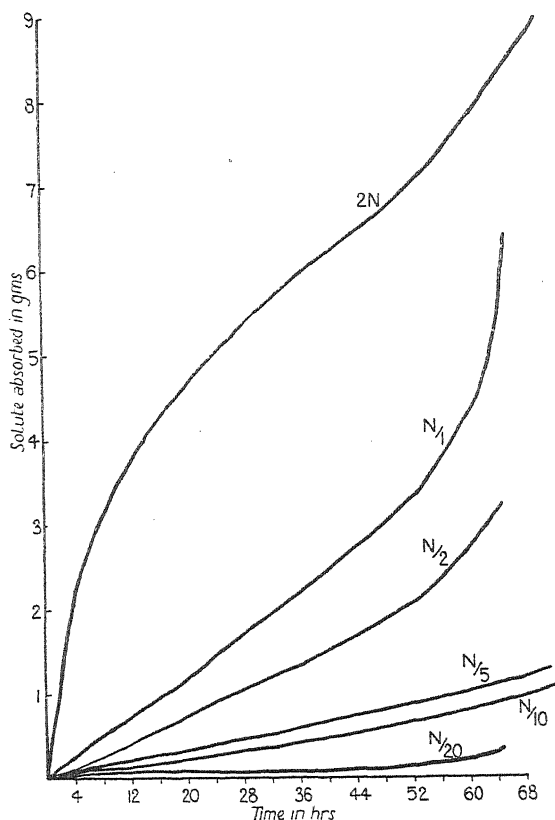


FIG. 3. Rate of absorption of sodium chloride from aqueous solutions of varying concentration in grams per 1,000 grams of seed.

takes place in the testa and its cuticular membranes. Clearly, however, the adsorptive capacities of the tissues of the endosperm and of the embryo must also affect the rate of absorption. Substances absorbed become physically inactive, and therefore the amount of solute that must diffuse into the seed in order to establish equilibrium on the two sides of the membrane of the seed will be greater the greater the adsorptive capacities of the starch for that particular compound. The absorption of the solute by the split seed was determined and compared with the absorption of the same compounds by intact seeds. The absolute amounts absorbed by the cracked seeds are naturally greater, but the relative amounts remain approximately the same, suggesting that the relative

absorption is determined partly by the tissues of the endosperm and embryo and partly by the seed-coat.

It would, therefore, seem that the rate of absorption is regulated by processes of adsorption. This view has been previously put forward by Brown and Tincker (5). These workers when working with certain organic compounds found that the rate of absorption of the solute is inversely proportional to the surface tension of the solution. More recently Gurewitsch has found that the diffusion of mercuric chloride across the seed-coat is accompanied by a rhythmic precipitation similar in appearance to the Liesegang rings. These observations suggest that the diffusion is taking place through colloidal membranes, in which case adsorption is probably operative. The present investigator, however, has been unable to confirm the observations of Gurewitsch.

The influence of adsorption in the earlier stages of absorption from solutions of electrolytes is further brought out by the relationship between absorption of the solute by the seed and the concentration of the solution. This relationship can be expressed within certain limits, by the well-known Freundlich adsorption equation. The form of this equation used in these investigations is

$$\frac{x}{a} = dc^{\frac{1}{n}}$$

where  $x$  = amount absorbed,

$a$  = surface area,

$c$  = final concentration of the external sol.

and ' $d$ ' and ' $n$ ' are constants; the value of ' $n$ ' is app. 2.

This equation does not cover absorption from solutions of high concentration. In Fig. 2 the absorption of the acid from the twice normal solution is anomalous. The low absorption of the solute from the solution is accompanied by a low absorption of water. There seems to be some connexion between the absorption of solute and the absorption of water.

A number of investigators have found that the higher the concentration the lower the absorption of water, this effect being due to the action of the semi-permeable membranes during absorption from solutions of increasing osmotic pressure. The retarding effect of the osmotic pressure of the solution will clearly vary with the imbibitional pressure that is developed by the endosperm. Such variations in the imbibitional pressure have been described by the present writer elsewhere (6).

The lower the absorption of water the less is the stretching effect of the endosperm on the seed-coat. Assuming that there is a connexion between the degree of stretching and the permeability, then the lower absorption of water from a solution of high concentration would lead to a lower permeability of the membranes of the seed-coat to the solute.

The degree of stretching of the seed-coat only becomes a limiting factor below a certain minimum which corresponds to a minimal absorption of water. Rippel has noted an increasing absorption of solute with increasing

TABLE I.  
*Absorption of Solute and of Water per 100 grm. Seed from Solutions of Varying Concentration.*

		NaCl.	H <sub>2</sub> O.
4 hours.	2 N	2.26	127
16	"	3.99	197
28	"	5.66	210
40	"	—	207
52	"	7.24	204
64	"	9.27	233
4	" N/1	—	165
16	"	—	246
28	"	2.00	265
40	"	2.59	277
52	"	3.71	276
64	"	6.59	290
4	" N/2	—	187
16	"	0.12	276
28	"	1.44	300
40	"	2.24	307
52	"	—	315
64	"	3.36	331
4	" N/5	—	185
16	"	—	303
28	"	0.49	332
40	"	—	371
52	"	0.69	376
64	"	1.02	380

absorption of water, and such he attributes to the breaking of the seed-coat consequent upon stretching. If such is the case the seeds that have been immersed in a weak solution for some 24 hours should, when dried, reabsorb water at approximately the same rate as seeds that have been cracked; whereas actually they do not. The explanation put forward in this paper that the stretching of the seed-coat increases the size of the inter-molecular pores seems a more probable explanation of the increased permeability. It would seem that in certain areas the molecular volume may affect the rate of absorption. Many organic compounds with complex molecules such as sugars and soluble proteins are excluded altogether. Molecular volume only seems to be operative in extreme cases such as those noted above. Organic compounds that are non-dissociated and have comparatively small molecules are more readily absorbed, such as the alcohols and phenols. It should be noticed, however, that even in such cases no absorption takes place until water is absorbed. No absorption of alcohol takes place when seeds are immersed in absolute alcohol.

SUMMARY.

1. The semi-permeable system is identified with the single layer of testa, and the cuticle-like membranes on its inner and outer surfaces.

2. Microchemical tests show that the testa is mucilaginous, and that the cuticle-like membranes contain more true fat than does cuticle of the normal foliage leaf.

3. The sequence during two types of absorption is described.

4. Quantitative estimations of the rate of absorption of various types of solute have been made.

5. Evidence is adduced to show that the cuticle-like membranes of the semi-permeable system carry electrical charges which affect the absorption of electrolytes.

6. It is shown that the adsorptive capacities of starch for any compound will affect the rate of absorption of that compound.

7. The importance of adsorption in the process of absorption of the solute is suggested by the fact that the relationship between absorption and concentration can be represented by the Freundlich absorption equation.

8. The effect of osmotic pressure of the solution on the absorption of the solute is shown.

9. It is suggested that molecular volume may affect the rate of absorption of compounds having very complex molecules, such as soluble carbohydrates.

*Conclusion.* The factors in the seed regulating the absorption of the solute would seem to be (1) electrical adsorption in the cuticle-like membranes, (2) mechanical adsorption in the testa and the tissues of the endosperm and embryo, (3) the imbibitional pressure developed in the endosperm, and (4) the size of the intermolecular spaces of the semi-permeable membranes.

The solute will react differently to each of the above according to its own particular properties. The extreme difficulty of formulating general laws to cover the absorption of all chemical substances is apparent.

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# Further Experimental Methods in connexion with the Use of the Katharometer for the Measurement of Respiration.

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With eight Figures in the Text.

## INTRODUCTION.

IN a previous paper on this subject Professor W. Stiles (1) and the writer described the application of the katharometer to physiological investigation for the purpose of measuring respiration. In that paper we endeavoured to put at the disposal of other workers the benefit of our very considerable experience with the instrument. In so doing it was necessary for us to point out where our experience was in agreement or at variance with that of previous workers. We have been informed that our comments on the experimental arrangement used by Gregory and Richards have been interpreted as suggesting that their experimental data are unsatisfactory. We would therefore point out that such an interpretation of our remarks was not intended and is not warranted. We were concerned solely with the development of the katharometer, and in indicating where experimental arrangements previously used were capable of improvement we were not calling in question the adequacy of such arrangements for the particular purpose for which they were employed. We also pointed out a number of sources of error in the use of the apparatus, which had not been mentioned by previous workers. It has been suggested to us that this might be interpreted as a failure on the part of these workers to take account of such sources of error. To avoid misunderstanding we would therefore point out that we realize that failure to mention a source of error did not necessarily mean that the writers were unaware of it or failed to correct it.

Subsequent work on plant respiration, carried out with the katharometer, the results of which will shortly be published, have convinced us of the unique value of the instrument for research of this nature. Its value has been still further enhanced by the development of a method for the automatic recording of galvanometer readings, with the result that

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continuous day and night records of the carbon dioxide output of respiring material may be obtained over periods of numbers of days.

It was from the beginning realized that the mere measurement of the carbon dioxide output of respiring tissue would furnish data of very limited value and that it would be desirable, if possible, to obtain corresponding measurements of oxygen intake. When working with a closed respiration chamber, the data for oxygen intake can be obtained if the chamber is fitted with some form of manometer for the purpose of measuring any changes that may occur in the pressure of the enclosed gas, as is done when working with the well-known Barcroft respiration apparatus. A simple manometer attachment for this purpose, which has proved satisfactory, will be described. The necessity, however, of taking visual readings of the manometer at intervals throughout an experiment, discounts to some extent the advantages gained by the automatic recording of the carbon dioxide output. In order to eliminate the necessity of taking such visual readings an electrically recording manometer has been devised. In this apparatus the katharometer principle is utilized, and the movement of the indicating liquid in the manometer detected by means of a galvanometer. This apparatus will give automatic records of pressure change with the recording device to be described below.

#### *Automatic Recording Camera.*

The usual type of galvanometer recording device is that in which a revolving drum carries a strip of paper on which a graph is drawn by means of a pen attached by a lever to the moving coil of the galvanometer. Such instruments are manufactured commercially, but none of them appear to be sufficiently sensitive for respiration measurements with the katharometer. The required degree of sensitivity can be obtained by using a reflecting galvanometer and replacing the scale with a revolving drum carrying a strip of bromide paper as described by Waller (2). This arrangement, while satisfactory from the point of view of sensitivity, possesses two serious disadvantages. Firstly, the apparatus must be kept in a dark room to avoid fogging of the sensitized paper, and secondly, a revolving drum of suitable size to carry a strip of bromide paper sufficiently long to deal with a total galvanometer deflexion of 50 cm. would be a somewhat unwieldy piece of apparatus to manipulate.

The apparatus about to be described records the galvanometer deflexion on a strip of bromide paper 50 cm. long and 3 cm. broad, which is held stationary in a dark slide. The source of light is an illuminated narrow slit, the image of which is projected on to the bromide-paper strip by the galvanometer mirror. The light illuminating the slit is switched on for a period of about 30 seconds at predetermined intervals by means of a clock fitted with adjustable contacts. Thus, each time the clock switches

on the light, the galvanometer deflexion is recorded on the bromide paper, and this may be arranged to take place at intervals of time, the lengths of which depend only on the capabilities of the clock.<sup>1</sup>

A general view of the camera used by the writer is shown in Fig. 1. It will be seen that it takes the form of a large box built of matchboarding and secured to the wall of the laboratory. It is made to accommodate two galvanometers, which are arranged to record simultaneously on the same strip of bromide paper carried in the dark slide marked A. Mounted on the front of the box, just above the dark-slide carrier, is an ordinary semi-transparent galvanometer scale (B, Fig. 1), and above this are two vertically placed pairs of galvanometer lamps, one pair for each galvanometer (C, Fig. 1). Of each pair of lamps the upper one carries the illuminated slit, the image of which is projected on the bromide paper in the dark slide, while the lower one is of the usual type having a vertical cross-wire, the image of which is projected on the scale. This lower lamp has a disc of orange-coloured gelatin between the electric bulb and the lens, thus enabling visual readings to be taken while a record is being made, without danger of fogging the bromide-paper strip. The positions of the recording lamps are so arranged that the respective images of their slits fall one above the other on the bromide strip, thus enabling one strip to carry two independent records. In order to allow of the inspection of the recording spots of light to be carried out, a second scale mounted in a wooden frame is provided and can be inserted in the dark-slide carrier.

The galvanometers stand on a heavy plate-glass shelf, which is firmly bracketed to the laboratory wall which forms the back of the box. The distance from this shelf to the front of the box carrying the lamps, scale, &c., is such as allows of accurate focusing of the images of the illuminated cross-wires and slits on the scale and bromide-paper strip.

Wires from the galvanometers are carried to terminals mounted on an ebonite strip which is screwed to the side of the box in a convenient place, for connecting up to the katharometer or other apparatus (D, Fig. 1). A light-tight door in the side of the box gives access to the galvanometers when adjustments are necessary.

The galvanometer lamps are constructed from old microscope eye-pieces and ordinary commercial 4-volt bicycle rear lamps. As it is advantageous to be able to regulate the brightness of the lights, each lamp is wired in series with a 6-ohm wireless valve filament rheostat. These rheostats, and also switches controlling the lights, are to be seen mounted on an ebonite panel below the dark-slide carrier in Fig. 1.

<sup>1</sup> Errors caused by slight shifting of the mill points of the galvanometers, due to the latter being continuously in circuit, have been eliminated by introducing an automatic switch which is also worked electrically by the clock, and which arranges that the circuits of the instruments are only closed for three minutes each time recording takes place.

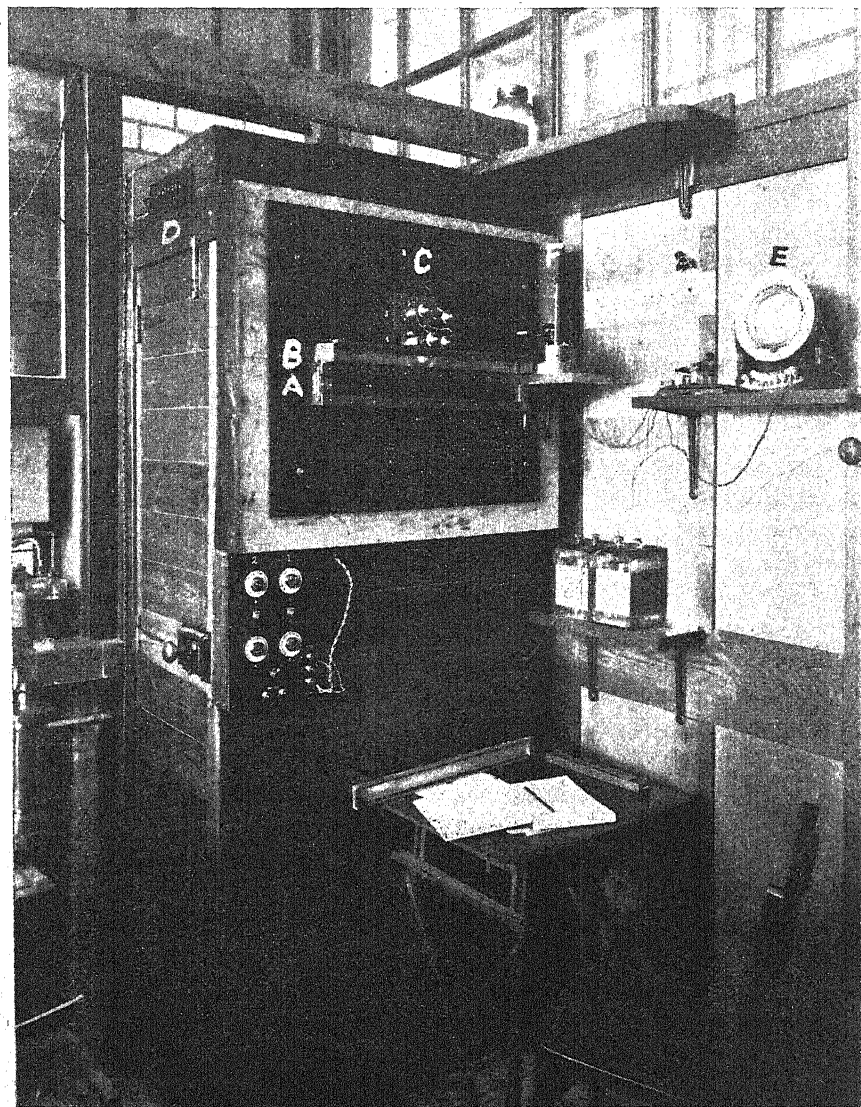


FIG. 1. Corner of laboratory showing the automatic recording camera. A, carrier with dark slide in position; B, galvanometer scale for visual reading; C, galvanometer lamps; D, terminals connecting with galvanometers; E, clock controlling recording lamps; F, clock controlling lever mechanism. For further description see text.

It will be readily understood that apparatus of this type is eminently suitable for making records when the galvanometer deflexion progresses in one direction only. For respiration work with the katharometer, where

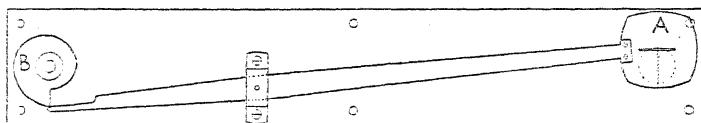


FIG. 2. For explanation see text.

a closed respiration chamber is used, it is entirely satisfactory, as the carbon dioxide concentration inside the chamber is continually increasing.

If apparatus is used in which the galvanometer deflexion undergoes reversal, means must be provided to prevent the images on the bromide paper becoming confused owing to their being superimposed. An additional attachment has been devised which overcomes this difficulty in a satisfactory way. This attachment is shown in Fig. 2. It will be noticed that a metal plate A, having a transverse slit cut in it, is mounted on a lever and is caused to travel in a vertical direction in front of the illuminated slit of one of the recording lamps. This movement is achieved by means of a cam, B, on which the end of the lever farthest from the lamp bears. The cam is rotated by a clock (F, Fig. 1) at speeds which can be varied at will from one hour to twelve hours per revolution. With this apparatus the marks recorded on the bromide paper, instead of having the form of horizontally arranged vertical lines, are obliquely arranged points. The obliquity of the arrangement of the points prevents their superposition when the direction of the galvanometer deflexion is reversed.

#### *A Simple Manometer Attachment.*

This device, as will be seen from an examination of Fig. 3, is a capillary manometer of the Barcroft type which can be attached to one of the side tubes of the katharometer plant chamber. As, when it is in use, both sides of the thread of indicating liquid communicate with closed chambers, changes in the pressure of the atmosphere which may occur during an experiment have no effect on the reading of the instrument. As the two closed chambers are entirely submerged in the water of the constant temperature bath, variations in the temperature of the air of the laboratory also have no effect on the readings. The actual manometer with its scale is above the surface of the water of the bath, so that readings can be taken without difficulty. In practice, the writer has found it most convenient to use a small piece of mirror for the purpose of examining the levels of the recording liquid.

The calibration of this manometer is readily carried out with the apparatus described on p. 591, as is done with the ordinary Barcroft apparatus, namely, by noting the changes in the level of the liquid in the two arms of the manometer which follow the introduction, or withdrawal of measured volumes of air into, or from, the katharometer plant chamber. The actual procedure when calibrating is described in detail on p. 591.

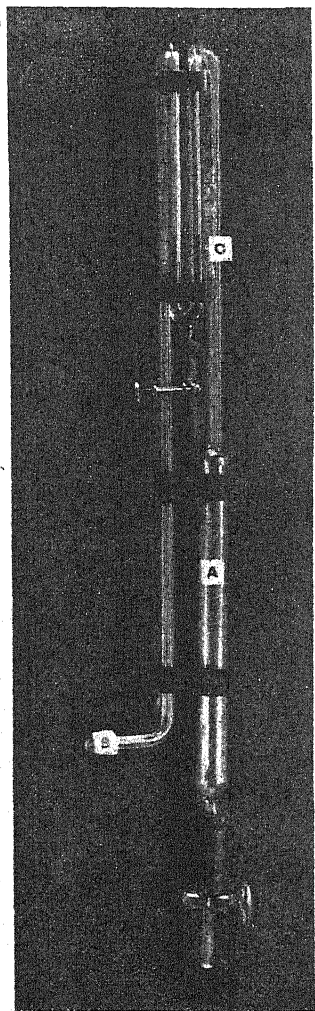


FIG. 3. Simple manometer. A, closed air chamber; B, tube for attaching to katharometer plant chamber; C, manometer with scale.

#### *An Electrically Recording Manometer.*

In the apparatus to be described the mechanism is the same in principle as that used in the katharometer. The general arrangement is shown in Fig. 4, from which it will be seen that the electrical circuit is in the form of a Wheatstone's bridge, in which the two 20-ohm manganin resistances  $V$  and  $V_1$  are balanced by two fine platinum wires A and H. One of these platinum wires A is stretched longitudinally in one arm of the manometer, while the other H is stretched in a straight glass tube. A current is passed through the system by means of the accumulator P, so that the temperature of the platinum wires is raised a few degrees above that of their containing glass tubes. The fluid contained in the manometer is liquid paraffin, and any change in the level of this fluid in the arm enclosing the heated platinum wire causes a change in the temperature of the wire. This, of course, is due to the high heat conductivity of the paraffin as compared with that of air. The change in the temperature of the platinum wire in the manometer arm causes a change in its resistance which in turn alters the electrical balance of the bridge circuit. This change is recorded by the galvanometer O. A change in the bridge balance can also be brought about by introducing a quantity of liquid into tube H, which contains the other platinum wire. By altering the level of the liquid in tube H, which we may call the control tube, the electrical balance of the instrument may be altered at will, and so the deflexion of

the galvanometer needle is altered. This change is recorded by the galvanometer O. A change in the bridge balance can also be brought about by introducing a quantity of liquid into tube H, which contains the other platinum wire. By altering the level of the liquid in tube H, which we may call the control tube, the electrical balance of the instrument may be altered at will, and so the deflexion of

the galvanometer O may be adjusted to meet any experimental requirements.

When this apparatus is used with the katharometer to record changes in pressure in the experimental plant chamber, the plant chamber is connected to one arm of the manometer at E. It will be noted that the other arm of the manometer connects to the closed chamber B. This chamber prevents variations in atmospheric pressure affecting the manometer during an experiment. A further point of importance is the fact that the composition of the gas mixture surrounding those parts of the platinum wires that are not immersed in paraffin must be kept constant. Changes in this gas will naturally introduce errors by causing the instrument to function as an ordinary katharometer. The only changes in this gas that can

occur under working conditions are those of water vapour concentration, and these are easily obviated by keeping the air in B and H saturated with water vapour. In general the same precautions are necessary in the use of this apparatus as in the use of the katharometer, and for details of these the reader is referred to the already mentioned paper dealing with that instrument (1).

The apparatus in its working form is shown in Figs. 5 and 6, and scale drawings of it are given in Fig. 7. It is mainly constructed of Monax glass, and comprises essentially the manometer AD, the control tube H, and the tube J containing the two 20-ohm balancing resistances. The manometer and control tubes are of capillary tubing of 1.5 mm. bore. The platinum wires, which are 0.001 in. in diameter are 16 cm. long, and are soldered to copper leads at their lower ends. The upper ends are soldered to spirals of No. 40 S.W.G. copper wire which act as springs to keep the platinum wires taut. As it is important that the platinum wires shall not touch the sides of their containing tubes, constrictions are formed in these tubes near their ends to ensure a central orientation of the wires (see Fig. 7). The wires are sealed into the tubes, as shown in the figure, by means of

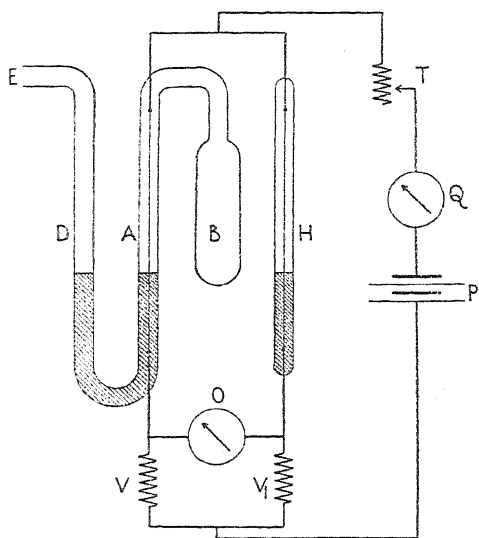


FIG. 4. Diagram of electrically recording manometer showing electrical circuit. AD, manometer; B, air chamber; H, control tube; V and  $V_1$ , 20-ohm balancing resistances; O, recording galvanometer; P, 4-volt accumulator; T, current-regulating rheostat; Q, milliammeter. For further explanation see text.

'Technico' sealing-wax. The balancing resistances  $v$  are of No. 30 S.W.G. silk-covered manganin wire; they are wound together in a single spiral and have their silk coverings impregnated with paraffin wax. The various leads are carried up to four terminals  $M$ , mounted on an ebonite cap  $L$  on top of tube  $J$ , two of these terminals being for connecting through a variable resistance and milliammeter to a four-volt accumulator, and two for connecting to the recording galvanometer. In the case of the instrument figured, a current of 65 milliamperes was used for heating the platinum wires. With this current, and using a D'Arsonval galvanometer having a resistance of 10 ohms and giving a deflexion of 60 mm. per microampere, and with an external series resistance of 100 ohms, a change of 1 mm. in the level of the paraffin in the two arms of the manometer gave a change in galvanometer deflexion of approximately 10 mm.

As in the case of the katharometer, this instrument is very sensitive to variations in the heating current, an increase in the heating current giving an increase in sensitivity. While working with the katharometer it was found that ordinary sliding rheostats were unsatisfactory for adjusting the heating current owing to variations occurring in the resistance of their contacts. This difficulty disappeared when the sliding rheostat was replaced by a specially constructed conductance box having very efficient contacts.<sup>1</sup> This current-regulating device was found to be quite satisfactory when used with the manometer.

In setting up the apparatus the required amount of liquid paraffin is introduced by suction into the manometer, and the galvanometer and battery circuits connected to their respective terminals. Glycerol is used in the control tube, as paraffin would cause trouble owing to its being absorbed by the rubber connexion  $R$  between the tube and the tap  $S$ . A quantity of this liquid is sucked into the control tube until the galvanometer deflexion is approximately zero. The control tube is then sealed by the tap  $S$  and the screw clip at  $U$ , after a small strip of wet filter paper has been introduced into tube  $U$ . The accurate adjustment of the galvanometer deflexion is then completed by means of the screw clip on the piece of thick-walled rubber tubing  $R$ . It is to be noted here that the atmosphere in the closed arm of the manometer must be kept saturated with water vapour. This is achieved by having a small quantity of water in the air chamber  $B$ .

The end  $E$  of the manometer tube may then be connected up to the katharometer plant chamber and the whole immersed in a constant temperature bath, so that only about 1 in. of the ebonite cap  $L$  projects above the surface of the water. The tube  $C$ , of course, leads above the surface of

<sup>1</sup> This conductance box was made by Messrs. Tinsley & Co. according to specifications supplied to them.



the water in the bath, and the tap in this tube is kept open until the whole apparatus has reached the temperature of the bath.

Calibration is carried out by means of apparatus identical with that used for calibration of the Barcroft respiration apparatus. The arrangement is clearly shown in Fig. 8, the apparatus being connected by means of the capillary tube H to the katharometer plant chamber G. By raising tube B, which contains the mercury, a known amount of air can be forced from the pipette A into the plant chamber.

In calibrating the manometer the procedure is as follows:

1. Close tap D
2. Turn taps E and F so as to connect H, A, and C.
3. Raise or lower B until the levels of the fluid in C and K are the same.
4. Read the level of the mercury in pipette A.
5. Read the manometer recording galvanometer.
6. Raise B about 1 cm.
7. Open and close tap D.
8. Readjust height of B until the level of the fluid in C is the same as in K.
9. Read the level of the mercury in pipette A.
10. Read the manometer recording galvanometer.

The differences between the readings of the mercury levels at stages 4 and 9 will give the amount of air introduced into the plant chamber G. The differences between the readings taken at stages 5 and 10 will give the deflexion of the recording galvanometer which corresponds with this increase in the amount of gas in the plant chamber.

Similar procedure should be followed in calibrating for reductions in pressure of the gas in the plant chamber, but, of course, in this case the mercury reservoir B must be lowered at stage 6 before tap D is opened and closed in order to *withdraw* a quantity of gas from the chamber.

#### *Calibration of the Katharometer for Air and Carbon Dioxide Mixtures.*

In the method previously described (1) for the calibration of the katharometer for air and carbon dioxide mixtures, respiring seeds of *Pisum sativum* were used as a source of carbon dioxide. When this method was employed, it was realized that, although it was satisfactory for a preliminary trying out of the instrument, there might be serious disadvantages accompanying its use as an actual experimental method. The first of these disadvantages is that where a number of peas are allowed to germinate in a confined space, there is danger of gases other than carbon dioxide being evolved, which would affect the katharometer. As an example of such a gas we have alcohol vapour, which can frequently be detected by its

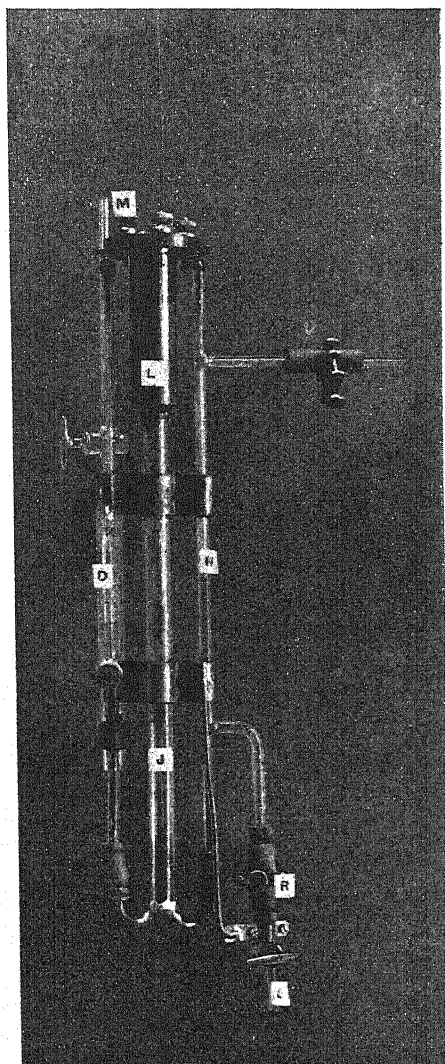


FIG. 5.

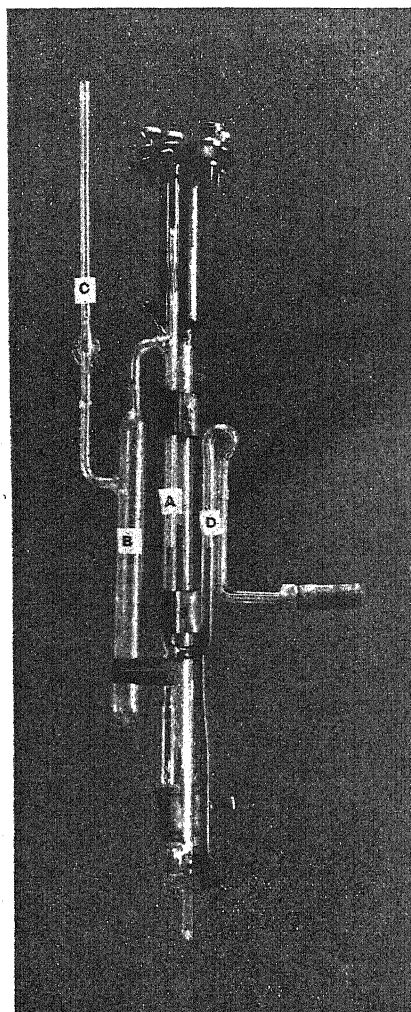


FIG. 6.

FIG. 5. Electrically recording manometer; side view showing D, manometer; H, control tube; J, tube containing balancing resistances. Compare Fig. 7A.

FIG. 6. Electrically recording manometer; front view showing manometer with air chamber. Compare Fig. 7B.

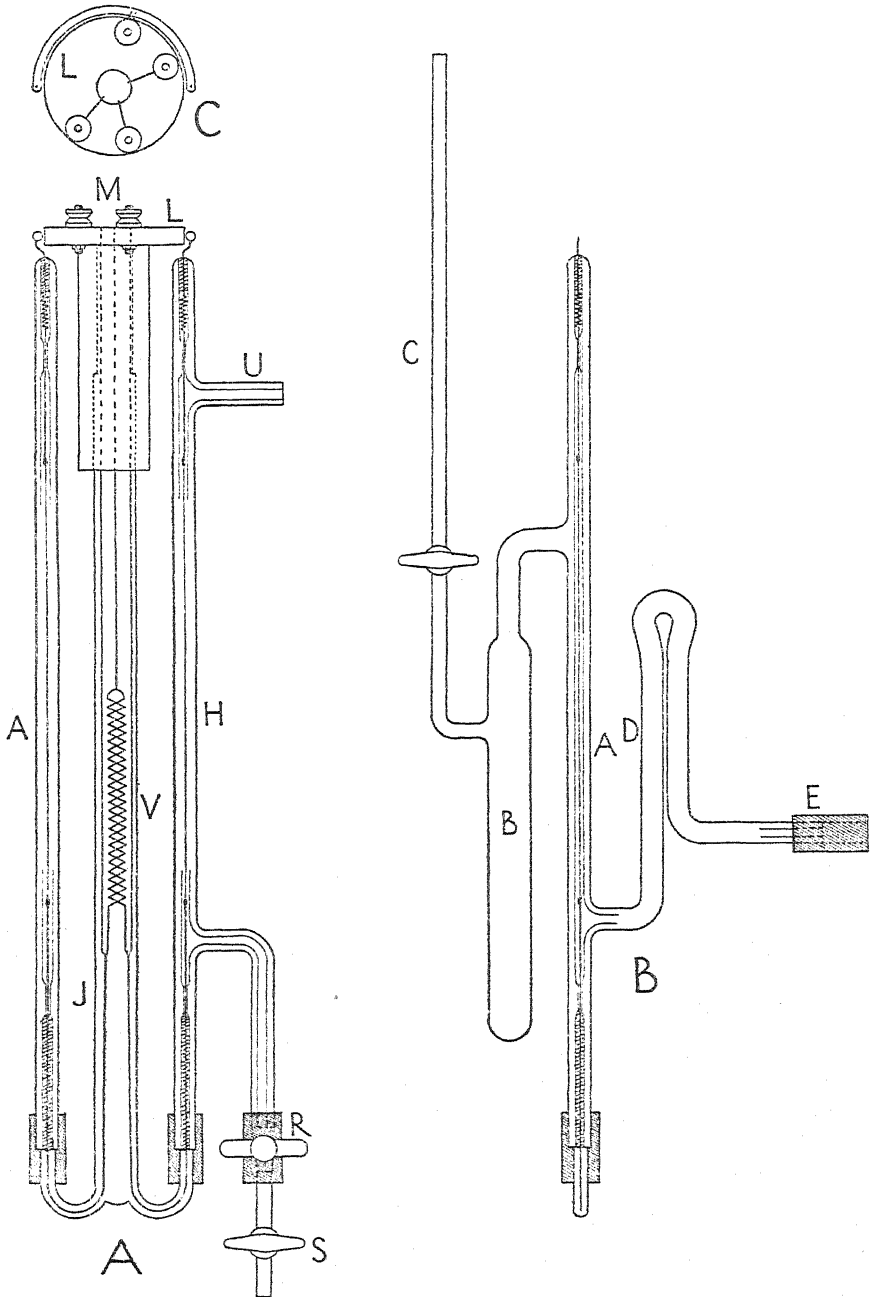


FIG. 7. Scale drawing showing constructional details of electrically recording manometer. A, side elevation, B, front elevation, C, plan of cap with terminals. AD, manometer; H, control tube; B, air chamber of manometer; J, tube containing 20-ohm balancing resistances (V); L, ebonite cap carrying terminals (M). Scale  $\frac{1}{2}$  full size. For further explanation see text.

smell when germinating peas are kept in a closed vessel. Secondly, the apparatus required for this method of calibration is complicated and unwieldy, and its use involves considerable disturbance of the katharometer as set up for actual respiration measurement. Thirdly, the work involved in calibration by this method is laborious and troublesome. It is to be noted, however, that if an investigation were being made on the respiration of germinating peas this method of calibration might be the most suitable one to use.

After a considerable amount of experimenting, the writer has found the method of adding to the air of the plant chamber measured quantities of pure carbon dioxide to be satisfactory for most purposes, in that it gives consistent results and is simple to carry out.

The apparatus is that shown in Fig. 8, and used for the calibration of the manometer as already described. For this method it is desirable to have a plant chamber fitted with four taps, of which one is connected by a capillary tube to the calibration apparatus, another to a capillary manometer, the remaining two being fitted with india-rubber tubes, one connecting to a suction-pump, and the other leading through a wash-bottle containing water to the outside air. The purpose of the manometer is to enable the readings to be corrected for increase in the pressure of gas in the plant chamber. A source of supply of pure carbon dioxide is connected to a wash-bottle of boiled distilled water, and thence to the tube J on the calibration apparatus. The katharometer plant chamber and the two wash-bottles are immersed in the constant temperature bath. The fluid in C (which for carbon dioxide calibration should be liquid paraffin or some other fluid which does not readily dissolve the gas) and the mercury in A are raised to the taps F and E respectively, and these taps are then turned so as to connect J and H only. The tap D on the plant chamber is then opened, together with the one leading to the suction-pump. The other two plant-chamber taps are closed, and the tube is removed from the suction-pump. Carbon dioxide is then passed through the apparatus until all the air in the system is swept out. The carbon dioxide stream is then stopped, the tap D is closed, and taps E and F are turned so as to connect A, C, H, and J. The mercury level in A and the liquid paraffin in C are then lowered so that the gas above them is now pure carbon dioxide, and tap F is turned so that E and C but not J are connected. Air is then drawn through the plant chamber via the wash-bottle by means of the suction-pump until the galvanometer shows that all the carbon dioxide has been swept from the plant chamber. The air-stream is now stopped, the tap connecting the plant chamber to the manometer opened, and the remaining two plant-chamber taps closed.

The galvanometer reading is noted and calibration proceeded with exactly as described on p. 591. Manometer readings are also taken after

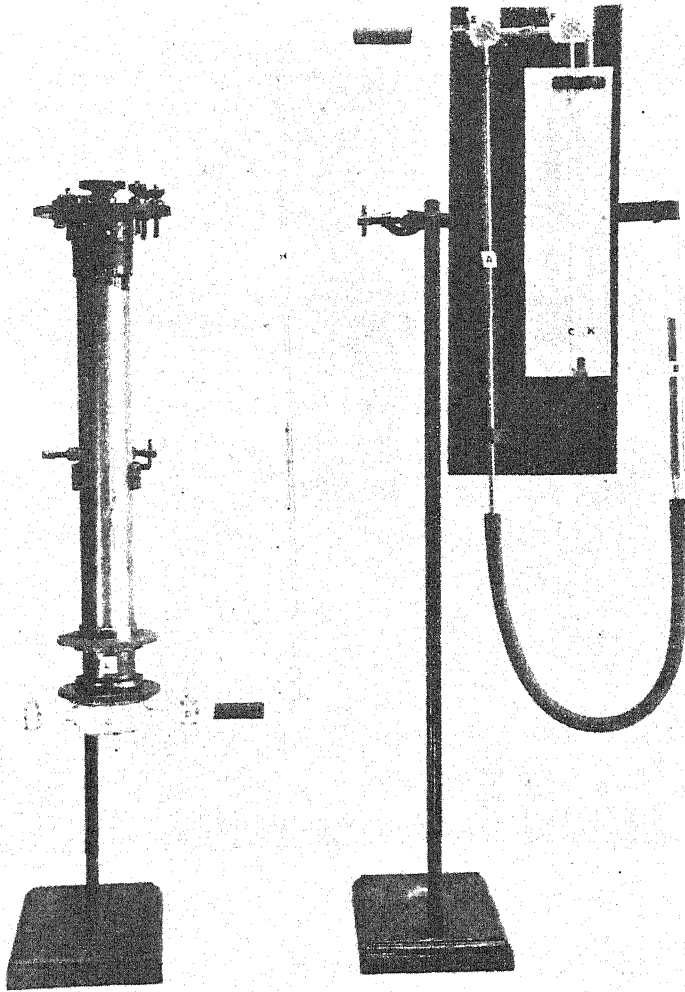


FIG. 8. Calibration apparatus. A, pipette graduated to 0.02 c.c.; B, mercury reservoir; CK, manometer; L, katharometer; G, katharometer plant chamber. For further explanation see text.

each addition of carbon dioxide to the air in the plant chamber. From these manometer readings correction of the galvanometer readings for pressure changes can be made.

In conclusion, it must be noted that both in the calibration of the manometer, as described on p. 591, and in the calibration of the katharometer, the volumes of gas introduced into the plant chamber are measured at atmospheric pressure and at laboratory temperature. These volumes must be corrected for normal temperature and pressure in order that all calibrations may be comparable.

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# Chemical Studies in the Physiology of Apples.<sup>1</sup>

## XIII. The Starch and Hemicellulose Content of Developing Apples.

BY

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With five Figures in the Text.

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### I. INTRODUCTION.

IN a study of the carbohydrate relationships of the apple it was assumed in the preliminary work (3) that the increase in the amount of alcohol-insoluble material might be taken as a measure of starch synthesis. A series of analyses of young apples during the growing season (May-September) of 1929, however, showed that a considerable increase in the amount of alcohol-insoluble material occurred before qualitative tests with iodine indicated the appearance of starch. This observation drew attention to the necessity for a method for the direct determination of starch, and to the need for further information as to the nature of the other constituents

<sup>1</sup> Thesis approved for the Degree of Doctor of Philosophy in the University of London.

of the alcohol-insoluble material. Determinations have hitherto been carried out on reducing power produced from the 'acid hydrolysable' or hemicellulose fraction by hydrolysis with 2.5 per cent. acid, but it is now generally recognized that the carbohydrates of the cell-wall are not all aldose condensation products, and, further, Murneek (32) has suggested that such 'hemicelluloses' may be important as storage or reserve substances in the apple. The occurrence and nature of hemicelluloses in the apple fruit, together with the change in the starch content during growth, have therefore been investigated.

The investigation has fallen into three parts:

(1) Elaboration of a method for the estimation of starch in apple tissue.

(2) Determination of starch and other polysaccharides in two varieties of apples throughout two growing seasons.

(3) Investigation of the nature of the easily hydrolysable polysaccharides other than starch or pectin, present in apple tissue.

## II. THE DETERMINATION OF STARCH IN APPLE TISSUE.

The usual method of extraction of starch from plant material is by enzyme hydrolysis, and in addition two methods of direct estimation without previous hydrolysis to reducing sugars have been described. Rask (48), working with cereals, treated the material with 20 per cent. HCl to extract the starch, precipitated the extracted starch with alcohol, and weighed the precipitate. Denny (16) found that for starch in melon seeds quantitative results with taka-diastrase did not agree with qualitative starch-iodide tests. He devised a method of extraction of the starch from the seeds by saturated calcium chloride solution, and the starch was then precipitated on the addition of iodine as the starch-iodide compound. Both these methods were tried with apple tissue, but in neither case was solution of the starch complete under the conditions described. A longer time for the extraction of starch by HCl was required than that advocated by Rask, and consequently a partial hydrolysis to reducing sugars had occurred before the alcohol was added.

Several workers have carried out estimations of starch in apple tissue. Neller and Overley (34) determined 'starchy material' in mature apples by the method of acid hydrolysis, realizing that some of the reducing sugar obtained may have been derived from a source other than starch. Gerhardt (20) and Murneek (31) have used ptyalin for the extraction of starch from dried, ground apple tissue, previously extracted with alcohol to remove the sugars and acid, and with 10 per cent. alcohol to remove dextrins. These workers hydrolysed the products of enzyme hydrolysis with 2.5 per cent. acid, a procedure open to the criticism that a destruction



of glucose probably occurs during the acid treatment. Murneek worked with flowers and immature fruits, and Gerhardt used material gathered at the normal picking date. Both workers obtained very variable results. This would appear to be due to the technique employed for the analysis, since the size of the samples used (100 spurs) would avoid any large differences due to sampling error.

Murneek, in his work on samples of very young fruits, found as much as 0.42 per cent. of starch present before the flowers were open, and the concentration increased to a value of 1.37 per cent. in the middle of June. These results are not in agreement with observations made by Howlett (26), nor with those made during the present investigation on Bramley's Seedling and Worcester Pearmain apples, when both qualitative and quantitative tests failed to detect starch in the fruit until the middle of June, when the apples were about  $\frac{3}{4}$  in. in diameter. Hydrolysis of starch by ptyalin carries with it the disadvantage that dextrin is among the products of hydrolysis, and that a further hydrolysis is necessary to convert this to reducing sugar.

The use of taka-diastrase was therefore examined by Widdowson (55), and was shown to give satisfactory results for pure starch, thus confirming the observation made by Davis and Daish (13) that glucose and maltose are the only products of hydrolysis. This result also appears to be confirmed by the work of Lehmann (27), who obtained a value of 100 per cent. of hydrolysed starch as glucose, provided that the starch was gelatinized by heating with water for forty minutes in a boiling-water bath, and provided that the hydrolysis was carried out at 37° at pH 4.9.

It was therefore decided to use a method of hydrolysis by taka-diastrase.

#### *Preparation of Material for Analysis.*

In preparing material for starch estimations the sugars must first be extracted with alcohol (see 2). This may be carried out on fresh tissue or on material which has been dried and ground. The latter method has been widely used, since it affords opportunity for obtaining a more homogeneous sample, but there appears to be no critical evidence to show that there is no change in starch occurring from enzyme action or other causes during the rather slow drying process. In this work, therefore, it was thought better to carry out the alcohol extraction on fresh material. The apple tissue was cut up and extracted as described by Haynes and Archbold (23) and the preliminary investigations were carried out as follows.

About 0.5 gm. of dried alcohol-insoluble apple residue containing starch was boiled for 30 seconds with 40 c.c. of water to emulsify the starch, cooled to room temperature, and 5 c.c. of freshly prepared 0.4 per cent. taka-diastrase solution, 2 drops of 5 per cent. acetic acid, and 0.2 c.c. of

toluene were added (6). The mixture was incubated at  $38^{\circ}$  for 24 hours, after which it was removed from the incubator and heated to boiling to destroy the enzyme. The residue was filtered off and washed with hot water, and a sample was treated with iodine solution and examined under the microscope. The starch appeared to be almost unattacked by the enzyme and remained chiefly inside the cells in granular form. It was thus clear that a more thorough disintegration of the tissue was necessary in order that the emulsification of the starch by boiling water should be complete. A fine powder obtained by grinding apple pulp previously dried at  $50^{\circ}$  and then extracting with alcohol was next tried, since it is almost impossible to grind the dried alcohol-insoluble residue owing to its tough and leathery nature. 0.5 gm. of the dried powder was hydrolysed with taka-diastrase as before. Large quantities of starch still remained unhydrolysed by the enzyme. Further experiments were carried out on the finely ground material, increasing the amount of enzyme up to 0.1 gm. per 0.5 gm. apple residue, increasing the time of preliminary boiling with water to gelatinize the starch, and increasing the time of incubation at  $38^{\circ}$  up to 48 hours. In no case was the hydrolysis of the starch complete. The effect of adding a further quantity of enzyme at the end of 24 hours and again incubating for 24 hours was also tried, but no better results could be obtained.

Since further disintegration of the tissue by mechanical means seemed impracticable, preliminary treatment with potassium oxalate in the cold was tried, in order to remove some of the pectin before the enzyme hydrolysis was carried out. It is obviously necessary to perform this extraction in the cold, since heating would convert some of the starch to the soluble form and this would be lost in subsequent filtration. It was hoped that this method would make it possible to use the residue obtained by alcoholic extraction of fresh material for the starch determinations. Qualitative tests showed that under these conditions starch was completely removed from the tissues by subsequent hydrolysis with taka-diastrase.

The method finally adopted was as follows. A weighed quantity of alcohol-insoluble residue (about 0.5 gm.) was shaken with 100 c.c. of a 1 per cent. solution of potassium oxalate for 8 hours in a mechanical shaker. The residue was then filtered off and washed free from oxalate with cold water. The oxalate extract was tested with iodine and with Fehling's solution to see if any starch had been extracted during the shaking process. A negative result was obtained in both cases.

The wet residue was washed into a glass mortar with 10 c.c. water, and could then be ground up into a very fine pulp. It was transferred to a 200 c.c. conical flask by means of 90 c.c. water, and the mixture was heated in a boiling-water bath for 30 minutes to gelatinize the starch. It was cooled to room temperature, and the enzyme hydrolysis carried out as

already described (p. 599), using 10 c.c. of a 1 per cent. solution of taka-diastase, 2 drops of 5 per cent. acetic acid, and a little toluene, and incubating at 38° for 24 hours.

Under these conditions the hydrolysis of the starch was complete, provided that the grinding of the wet tissue before hydrolysis had been sufficiently thorough. In all subsequent determinations the residue remaining after enzyme hydrolysis was tested with iodine and examined under the microscope in order to ensure that the hydrolysis was complete. If any blue coloration was observed, the determination was discarded.

The filtrate containing glucose and maltose produced by hydrolysis of the starch was diluted to 500 c.c. and the sugar in 5 c.c. estimated by oxidation with alkaline ferricyanide and hypiodite. The quantity of each sugar present was calculated from simultaneous equations, and hence the amount of starch. The details of the methods used for the sugar estimations are those described by Widdowson (55).

A 'blank' determination was carried out on 0.5 gram. alcohol insoluble residue containing no starch, and the ferricyanide and iodine equivalents for this solution subtracted from the values for the residue containing starch before calculation of results.

*Effect of Clearing Solutions obtained by the Hydrolysis of Starch  
in Apple Tissue.*

It was found that the solutions after hydrolysis were brown in colour, so it was thought necessary to investigate the effect of clearing the solutions with basic lead acetate and sodium phosphate, since it has been shown that colouring matter may affect the iodine and ferricyanide values (5, 55).

No loss of sugar occurs if glucose, maltose, or hydrolysed starch solutions are cleared with basic lead acetate and sodium phosphate (55), so that any loss of reducing power which occurs on clearing solutions obtained by hydrolysing the starch in apple residue will be due to the removal of some substance other than sugar from the solution.

The following materials were used to test the effect of clearing:  
(1) 0.5 gram. residue containing starch; (2) 0.5 gram. residue containing no starch; (3) 0.5 gram. residue containing no starch + 0.3 gram. purified starch; (4) 'blank' determination for taka-diastase.

The enzyme hydrolyses were carried out as before, and the hydrolysed solutions were filtered and diluted to 500 c.c. Two hundred c.c. of each were cleared with 1 c.c. basic lead acetate and 3 c.c. sodium phosphate, diluted to 250 c.c. and filtered. The brown colour was completely removed by this procedure. A second 200 c.c. of each were diluted to 250 c.c. without clearing.

In the case of the 'blank' determination for taka-diastase filtration of the cleared solution was rapid, and a clear solution was obtained. This had previously been found to be the case with cleared solutions obtained from the hydrolysis of purified starch. Cleared solutions obtained from starchy or non-starchy residues, however, only filtered extremely slowly, and it was necessary to filter several times before a clear solution was obtained. The addition of a little sodium fluoride before clearing, as suggested by Horton (25), was found to be of no benefit, nor was there any improvement if the cleared solutions were left to stand overnight before filtering.

The sugar in 5 c.c. of each of the cleared and uncleared solutions was estimated by oxidation with alkaline ferricyanide and hypiodite.

It has already been shown (55) that taka-diastase is not precipitated by basic lead acetate and sodium phosphate, and this observation was confirmed by the fact that there was no difference between the values for the cleared and uncleared solutions by either method of oxidation for the taka-diastase 'blank' in these experiments. A decrease in the values both for the ferricyanide and the iodine oxidations, however, resulted from the clearing of extracts prepared from apple pulp.

The results are shown in Table I.

TABLE I.

*Effect of Clearing on Solutions obtained from Apple Residues.*

Results expressed as c.c. N/100 thiosulphate  $\equiv$  ferricyanide or iodine reduced by 5 c.c. solution.

	Thiosulphate equivalent to—			
	Ferricyanide reduced.		Iodine reduced.	
	Cleared. c.c.	Uncleared. c.c.	Cleared. c.c.	Uncleared. c.c.
1. Residue containing starch .	5.26	5.55	1.85	1.92
2. Residue containing no starch	0.68	1.04	0.26	0.31
3. Residue containing no starch + 0.3 grm. starch . . .	3.47	3.82	1.08	1.15
4. Blank for taka-diastase .	0.68	0.70	0.26	0.26

These results indicate that there is some substance other than starch which is extracted by hot water from the alcohol-insoluble apple residue which is oxidized by alkaline ferricyanide and hypiodite, and which is apparently completely precipitated by basic lead acetate and sodium phosphate.

As a result of these determinations it was concluded that all solutions obtained by the hydrolysis of starch in apple tissue must be cleared before the sugars in them are estimated.

*Examination of Alcohol-insoluble Apple Residue for Dextrin.*

Gerhardt (20) has found amounts of dextrin in apples varying from 0.10 per cent. to 0.57 per cent. of the fresh weight of the tissue by extracting the alcohol-insoluble material with 10 per cent. alcohol at 50°. If dextrin is present at any stage during the development of the fruit it would probably be extracted from the residue during the shaking with potassium oxalate solution, and would therefore be lost in the present method of analysis.

Murneck (30) used water at room temperature for the extraction of dextrin from tomatoes, and Davis and Sawyer (14) have shown that water at 38° is suitable for extracting dextrin from the leaf of the potato. Tests showed that starch is quite insoluble in water at 38°. Some alcohol-insoluble apple residue containing starch was accordingly extracted with water at 38° for 16 hours and the residue was then filtered off. The filtrate gave no blue or red colour with iodine, and on the addition of alcohol no precipitate of dextrin was produced. Therefore dextrin, if present at all, could only occur in very small amounts.

One gramme of each of a number of samples of alcohol-insoluble residues from Bramley's seedling apples at different stages of development were next tested for dextrin. The samples were extracted with 100 c.c. of water at 38° for 24 hours. The residues were filtered off and washed, and 5 c.c. of 1 per cent. taka-diastase solution, 2 drops of 5 per cent. acetic acid, and a little toluene were added to each filtrate and the solutions were again left at 38° for 24 hours. The flasks were removed from the incubator, the solutions heated to boiling, and cleared with 1 c.c. basic lead acetate and 3 c.c. sodium phosphate, diluted to 250 c.c. and filtered. The cleared solutions were difficult to filter, indicating that the colloidal substance which renders difficult the filtration of the cleared solutions obtained from the hydrolysis of starch in apple residue is extracted with water at 38°.

The reducing power of 5 c.c. of all solutions was then estimated by oxidation with alkaline ferricyanide. The maximum concentration of apparent dextrin, calculated as glucose, which was reached at any time was 0.02 per cent. of the fresh weight, which is much less than the sampling error of the starch determinations. This value coincided with the maximum starch value, and it seems likely therefore that there is never more than a trace of dextrin present in tissue from the varieties of apples under consideration.

*Investigation of Sampling Error on the Starch Determinations.*

In order to determine the sampling error on results for the percentage of starch in apple tissue, five portions of the same sample of residue were

hydrolysed with taka-diastrase in the ordinary way, and the percentage of starch in each was calculated.

The results are shown in Table II, which gives the mean of five results and their standard error.

TABLE II.

*Starch Content of Uniform Samples of Dried Apple Residue after Alcohol Extraction.*

	% starch in residue.	% starch in fresh weight.
1.	35.54	1.262
2.	31.57	1.121
3.	33.49	1.189
4.	34.09	1.210
5.	33.27	1.181
Mean value	$33.59 \pm 1.41$	$1.193 \pm 0.051$

*Specificity of Taka-diastrase.*

In view of the question which has arisen during the past few years as to the specificity of enzymes for any one substance (see 22, 52) and the fact that Wohlgemuth (56) has shown that taka-diastrase may contain amylase, trypsin, rennet, erepsin, lipase, and haemolysin, while Nishimura (36) has further identified saccharase, protease, lactase, catalase, inulase, sulphatase, and amidase in preparations of the enzyme, it cannot be assumed that starch is the only substance present in apple tissue which is acted on by taka-diastrase to give reducing sugars. A rigid test for the specificity of taka-diastrase is difficult to obtain, but a careful investigation of its action on apple tissue has been made, the results of which indicate that an action on any substance other than starch in the tissue is extremely unlikely.

The alcohol-insoluble residues from apples just too young to contain starch, and from fruits immediately after its disappearance, were treated with taka-diastrase, under the usual conditions for starch hydrolysis, and the extracts were cleared and the reducing power of the solutions determined. It was found that there was no appreciable oxidation by the ferricyanide or iodine, the thiosulphate titrations agreeing within 0.02 c.c. with the titration for the blank determinations for taka-diastrase. This indicates that starch is the only substance in apple tissue which is hydrolysed by taka-diastrase, since it is unlikely that there is a reducing substance, or some substance which is acted upon by taka-diastrase to give a reducing substance, which appears and disappears simultaneously with the starch, and which is not removed from the solution by clearing with basic lead acetate and sodium phosphate.

Further evidence of the reliability of the method, involving hydrolysis

of starch in apple tissue by taka-diastase, was obtained by carrying out estimations on purified maize starch with 0.5 gm. of residue containing no starch added. The mixture was shaken with potassium oxalate and hydrolysed with taka-diastase in the usual way, filtered, cleared, and the sugar in the solution determined. Results which were accurate to within 1 per cent. were obtained in every case. They are shown in Table III.

TABLE III.

*Hydrolysis with Taka-diastase of Purified Maize Starch to which had been added 0.5 gm. Alcohol-insoluble Residue containing no Starch.*

	Starch taken. gram.	Starch found. gram.	Starch found. %
1.	0.2439	0.2435	99.84
2.	0.1579	0.1568	99.30
3.	0.3670	0.3659	99.70

A series of hydrolyses of starch-containing residues by ptyalin has also been carried out. Ptyalin has been used satisfactorily for the hydrolysis of starch in plant materials by a number of workers (19), (20), (31), (53). The results obtained by Tottingham and Gerhardt (53) suggest that in the tissue used by them either the hydrolysis of the starch by taka-diastase was incomplete, or some material other than starch was attacked by ptyalin. The further hydrolysis necessary to convert the dextrin produced by ptyalin to reducing sugars has generally been carried out by boiling with acid. In order to avoid destruction of glucose during acid hydrolysis, taka-diastase was used in the present instance as the second hydrolysing agent.

*Hydrolysis of Starch by Ptyalin.*

Determinations of pure starch by hydrolysis with ptyalin were found to give results which were within 1 per cent. of the weight of starch taken. The following method was used: 0.3 gm. starch was weighed out into a 250 c.c. conical flask, 100 c.c. water were added, and the mixture was heated in a boiling-water bath for half-an-hour. It was then cooled to room temperature, 2 c.c. freshly collected saliva and a little toluene were added, and the flask was incubated at 38° overnight. It was then removed from the incubator and the contents heated to boiling, cooled, and 10 c.c. 1 per cent. taka-diastase, 0.05 c.c. acetic acid, and a little toluene added. The flask was again placed at 38° for 24 hours, and, after heating to boiling, cooled, cleared with 2 c.c. basic lead acetate and 6 c.c. sodium phosphate, diluted to 500 c.c. and filtered, and the sugar in 5 c.c. estimated. A second estimation was carried out using 6 c.c. of saliva added in amounts of 2 c.c. at 8-hour intervals. Blank determinations were carried out on

2 c.c. ptyalin + 10 c.c. taka-dia-*stase*, 6 c.c. ptyalin + 10 c.c. taka-dia-*stase*, and 10 c.c. taka-dia-*stase* alone, and a control determination using 0.3 gm. starch hydrolysed with 10 c.c. 1 per cent. taka-dia-*stase* without previous hydrolysis with ptyalin. The results are shown in Table IV.

TABLE IV.

*Hydrolysis of Purified Maize Starch by Ptyalin followed by Taka-dia-*stase* and by Taka-dia-*stase* alone.*

Results expressed as N/100 thiosulphate  $\equiv$  ferricyanide or iodine reduced by 5 c.c. solution.

	Thiosulphate equivalent to—		
	Ferricyanide reduced. c.c.		Iodine reduced. c.c.
Starch hydrolysed 6 c.c. ptyalin + 10 c.c. taka-dia- <i>stase</i> . . . . .	11.39		3.94
'Blank' for " " " " " "	1.42		0.52
Starch hydrolysed 2 c.c. ptyalin + 10 c.c. taka-dia- <i>stase</i> . . . . .	10.00		3.45
'Blank' for " " " " " "	1.40		0.52
Starch hydrolysed 10 c.c. taka-dia- <i>stase</i> .	10.33		3.58
'Blank' for " " " " " "	1.40		0.52
Hydrolysing agent.	Starch taken. gram.	Starch found. gram.	Starch found. %.
6 c.c. ptyalin followed by 10 c.c. taka- dia- <i>stase</i> . . . . .	0.3252	0.3246	99.82
2 c.c. ptyalin followed by 10 c.c. taka- dia- <i>stase</i> . . . . .	0.2790	0.2800	100.36
10 c.c. taka-dia- <i>stase</i> . . . . .	0.2916	0.2904	99.59

*Hydrolysis of Starch in Apple Tissue by Ptyalin.*

The starch in a number of samples of alcohol-insoluble apple residues was then estimated both by hydrolysis with ptyalin followed by taka-dia-*stase*, and by hydrolysis with taka-dia-*stase* alone. For the determinations using ptyalin, the residue after the potassium oxalate treatment was ground and incubated with 2 c.c. of saliva at 38° overnight, and the mixture was then heated to boiling and the residue was filtered off and washed with hot water. Ten c.c. 1 per cent. taka-dia-*stase* were added to the filtrate and the mixture again incubated at 38°. After this second hydrolysis the enzyme was destroyed by boiling, and the solution cleared and the sugars determined as already described. In every case the residue after the ptyalin hydrolysis was tested for starch by iodine, but the removal of the starch was always found to be complete. Blank determinations were carried out on residue containing no starch, by both methods of hydrolysis. It was found that the 'blank' titration for ptyalin + taka-



diastase agreed with that for taka-diastase alone, indicating either that ptyalin is completely precipitated by lead acetate, or that it is not oxidized by alkaline ferricyanide or alkaline iodine.

The results of the determinations are shown in Table V.

TABLE V.

*Hydrolysis of Starch in Apple Residues with Ptyalin followed by Taka-diastase, and with Taka-diastase alone.*

	% starch in residue.	
	Hydrolysed by ptyalin followed by taka-diastase.	Hydrolysed by taka-diastase alone.
1.	16.05	16.23
2.	30.39	29.30
3.	36.16	36.81
4.	37.45	38.80
5.	32.05	31.03

Comparison of the two sets of results shows that the difference between any two results is never greater than the standard error, and therefore not significant. This indicates that the specific actions of taka-diastase and ptyalin on apple tissue is the same, and that apple tissue contains no insoluble substance other than starch, which is attacked by one enzyme but not by the other to give reducing sugars. A possible source of error would be the extraction of a water-soluble material during gelatinization, which would remain in the filtrate to which the taka-diastase is added, after ptyalin hydrolysis. From evidence to be presented, however (p. 625), it seems certain that no error is introduced in this way.

It has therefore been assumed that taka-diastase may be used for the quantitative estimation of starch in apple tissue.

### III. ANALYSIS OF ALCOHOL-INSOLUBLE MATERIAL OF BRAMLEY'S SEEDLING AND WORCESTER PEARMAN APPLES.

It has already been stated that this investigation of the hemicelluloses in the alcohol-insoluble material of apples was undertaken as part of a more complete study of the chemistry of the developing fruit which was being carried out in this laboratory (4), (18).

The present work was intended to supply quantitative results for the amount of starch in apple tissue, and further to identify and estimate other readily hydrolysable substances which might be present, since it has been suggested that pectin and allied compounds might play a part in supplying the substrate for respiration in the mature fruit.

Analyses of the alcohol-insoluble material were carried out during

two seasons, 1929 and 1930, on two varieties of apples, Bramley's Seedling and Worcester Pearmain, from the time of petal fall until some time after the normal gathering date. In addition, in 1929, samples of both varieties of apples were stored at 1° C., and determinations were made on samples withdrawn from store at frequent intervals. Samples for analysis during growth and storage were gathered from the same trees in both varieties of apple.

In 1929 the samples of Bramley's Seedling apples consisted of two apples from each of 13 trees, but, since these trees failed to bear in 1930, 2 apples were taken from each of 14 trees on a plot near by. In the case of the Worcester Pearmain apples, 2 apples from each of 10 trees were collected in 1929, and from each of 11 trees in 1930, these 11 trees including the 10 which had been used during the previous year.

With the exception of the first three collections, the material in 1929 was sent by post and the analysis was begun on the day following picking. In 1930 the samples were collected during the morning and the analysis begun during the same afternoon in every case. This second method resulted in a marked diminution of scatter in the values obtained by the analyses, due either to the more careful gathering or to the decrease in the time between the collection of the fruit and the commencement of the analysis.

Since it has been shown by Carré (10) that pectin is removed from apple tissue by N/75 acid, a preliminary trial was made to find how this strength of acid would affect the starch in the tissue. It was found that the starch was completely removed by this treatment, probably as a mixture of dextrins and reducing sugars. The investigation was therefore confined in the first instance to that fraction which is removed by N/75 acid from alcohol-insoluble material. It was found that there was no marked difference in the amount extracted, whether N/75 or N/20 acid was used, but it is probable that the more concentrated acid (2.5 per cent.), which is usually employed to convert starch to reducing sugars, has some action on the more stable components of the tissue.

The residues which remained after acid hydrolysis were weighed, so that a measure of very easily hydrolysable material could be obtained by difference, and hence a knowledge of how far this was accounted for by starch and pectin could be gained.

#### *Methods of Estimation.*

##### *Pectin and residue insoluble in N/75 HCl.*

About 1 gram. of alcohol-insoluble apple residue was weighed out and boiled with 100 c.c. N/75 HCl for 3 hours under a reflux condenser, filtered off, and boiled with a further 100 c.c. of the acid. This process

was repeated until no appreciable quantity of pectin was extracted; three boilings were found to be sufficient in every case (see 10). The final filtration was carried out through a weighed filter paper and the residue and paper were thoroughly washed with boiling water, dried in the oven at  $100^{\circ}$  and weighed. It has already been pointed out that starch is completely extracted from the residue by this treatment, so that the weight of residue insoluble in  $N/75$  HCl gives the weight of pectin-free and starch-free material.

The united filtrates and washings were neutralized and diluted to 500 c.c. Two aliquots of 200 c.c. were measured out, and the precipitation of pectin as calcium pectate was carried out, as described by Carré and Haynes (11). The calcium pectate was filtered off through fluted filter papers, and washed with boiling water until free from chloride. The precipitate was then washed into a beaker, boiled with distilled water, and again filtered. This process was repeated until, on further boiling, the filtrate contained no chloride. The final filtration was carried out through a weighed Gooch crucible, and the precipitate was dried at  $100^{\circ}$  and weighed.

#### *Starch.*

The starch determinations were carried out, as described on p. 600. 0.5 gram. dry alcohol-insoluble residue was shaken with potassium oxalate, washed, ground, gelatinized with water, and hydrolysed with 10 c.c. 1 per cent. taka-diastase, and the sugars in the cleared hydrolysate estimated by oxidation with alkaline ferricyanide and by hypiodite.

Tables VI, VII, VIII, and IX give the results of the analyses of the alcohol-insoluble residues from young Bramley's Seedling and Worcester Pearmain apples during two growing seasons. Values for starch, pectin as calcium pectate, and residue insoluble in  $N/75$  HCl (mainly cellulose) are shown, the results being expressed as percentage in the fresh weight of the apple tissue.

It will be evident from the tables that the sum of the starch, pectin as calcium pectate, and residue insoluble in  $N/75$  HCl only accounts for about 50-70 per cent. of the alcohol-insoluble material. It is clear, therefore, that the remaining 30-50 per cent. of the residue consists of material other than starch or pectin which is extracted by very dilute acid, and which remains in the extract after the pectin is precipitated. This readily hydrolysable fraction is present in the fruit at all stages of development. Since subsequent work has shown that the substances other than starch or pectin which are extracted by  $N/75$  acid belong to the type of compounds known as hemicelluloses, this fraction is referred to as hemicelluloses both in the tables and in the graphs.

TABLE VI.

*Analysis of Alcohol-insoluble Material of Developing Fruit of Bramley's Seedling Apples during 1929.*

Results expressed as % in fresh weight of apple tissue.

	Days from May 25.	Mean weight of one apple. grm.	Alcohol-insoluble residue.	Starch.	Pectin as Ca pectate.	Residue insoluble in N/75 HCl.	Hemicelluloses.	Hemicelluloses as % of alcohol-insoluble residue.
May	1	0.152	17.64	None	2.274	6.728	8.638	48.97
June	11	0.470	7.91	"	1.141	3.345	3.423	43.28
	22	2.66	5.25	0.369	0.683	2.648	1.550	29.53
	33	7.69	3.85	0.397	0.604	1.730	1.120	29.08
July	42	19.58	3.96	0.620	0.375	1.515	1.450	36.62
	54	(39.00)	4.03	0.701	0.584	1.419	1.325	32.89
	66	(56.00)	4.36	0.930	0.517	1.084	1.829	41.96
Aug.	81	61.20	4.16	1.213	0.547	1.027	1.373	33.00
	93	62.30	3.70	1.014	0.497	0.902	1.287	34.78
Sept.	108	92.3	3.12	0.690	0.492	0.795	1.143	36.63
	124	94.1	2.54	0.370	0.521	0.769	0.900	35.16
Oct.	150	118.5	2.12	0.035	0.504	0.715	0.866	40.86
Nov.	164	118.5	2.14	None	0.519	0.853	0.768	35.89

\* The values for hemicelluloses are obtained by subtracting the sum of the values for the pectin, starch, and residue insoluble in N/75 HCl from the values for the alcohol-insoluble residue.

TABLE VII.

*Analysis of Alcohol-insoluble Material of Developing Fruit of Bramley's Seedling Apples during 1930.*

Results expressed as % in fresh weight of apple tissue.

	Days from May 27.	Mean weight of one apple. grm.	Alcohol-insoluble residue.	Starch.	Pectin as Ca pectate.	Residue insoluble in N/75 HCl.	Hemicelluloses.	Hemicelluloses as % of alcohol-insoluble residue.
May	1	0.111	11.38	None	0.980	5.815	4.700	41.30
	4	0.247	7.96	"	0.719	3.912	3.329	41.82
	7	0.572	6.75	"	0.773	3.676	2.300	34.08
June	10	0.965	6.15	"	0.695	3.641	1.814	29.51
	16	2.239	4.66	"	0.590	2.574	1.496	32.10
	22	5.778	4.17	0.118	0.547	2.273	1.232	29.56
July	29	13.312	3.47	0.124	0.538	1.677	1.132	32.61
	38	24.885	3.57	0.377	0.548	1.406	1.239	34.70
	49	37.642	3.59	0.840	0.539	1.220	0.990	27.58
Aug.	63	54.357	3.53	1.031	0.498	1.121	0.879	24.90
	77	78.500	3.55	1.261	0.504	0.953	0.831	23.42
	92	87.94	3.51	1.463	0.538	0.872	0.636	18.13
Sept.	105	101.89	3.02	1.002	0.570	0.756	0.692	22.91
	119	123.10	2.59	0.700	0.549	0.710	0.630	24.33
Oct.	134	138.17	1.96	0.135	0.525	0.665	0.636	32.43
	148	150.68	1.73	None	0.519	0.643	0.568	32.85
Nov.	155	134.65	1.68	"	0.509	0.618	0.552	32.86
	163	139.07	1.69	"	0.497	0.622	0.571	33.78

TABLE VIII.

*Analysis of Alcohol-insoluble Material of Growing and Stored Fruit of Worcester Pearmain Apples during 1929.*

Results expressed as % in fresh weight of apple tissue during growth and % in original fresh weight during storage.

	Days from May 25.	Mean weight of one apple. gm.	Alcohol-insoluble residue.	Starch.	Pectin as Ca pectate.	Residue insoluble in N/75 HCl.	Hemicelluloses.	Hemicelluloses as % of alcohol-insoluble residue.
June	11	0.746	8.10	None	1.145	4.002	2.953	36.46
	22	1.71	6.58	0.342	0.855	3.328	2.055	31.23
	33	5.16	5.48	0.766	0.737	2.022	1.956	35.69
July	42	11.45	5.46	0.911	0.465	1.728	2.356	43.15
	54	(18.00)	7.00	1.766	0.448	1.872	2.914	41.63
	66	(28.00)	6.56	2.031	0.767	1.554	2.208	33.66
Aug.	81	45.0	5.63	1.899	0.720	1.191	1.820	32.33
	93	49.7	4.04	0.964	0.568	0.955	1.553	38.44
	115*	72.2	2.89	0.477	0.586	0.743	1.084	37.52
Sept.	121	"	2.79	0.283	0.619	0.776	1.112	39.85
	128	"	2.64	0.231	0.577	0.786	1.047	39.65
Oct.	135	"	2.49	0.130	0.561	0.816	0.982	39.44
	149	"	2.34	0.018	0.557	0.843	0.922	39.40
Nov.	163	"	2.43	0.041	0.638	0.837	0.914	37.60
	177	"	2.46	None	0.572	0.851	1.037	42.15

\* Apples gathered and placed in cold store at 1° C at day 115.

TABLE IX.

*Estimations of Starch in Growing Fruit of Worcester Pearmain Apples during 1930.*

Results expressed as % in fresh weight of apple tissue.

	Days from May 27.	Mean weight of one apple.	Alcohol-insoluble residue.	Starch.
June	22	4.276	5.68	None
	29	6.203	5.21	0.213
July	38	11.223	5.49	0.891
	49	21.773	5.72	1.676
	63	38.00	5.46	2.010
August	77	56.57	5.06	1.963
	92	72.64	4.17	1.294

Concentration curves for the alcohol-insoluble residue, starch, pectin, residue unhydrolysed by N/75 HCl, and hemicelluloses in Bramley's Seedling apples throughout one growing season (1930) are given in Fig. 1. The curves for the other sets of analyses are all of a similar type.

The concentration of alcohol-insoluble residue decreases rapidly during

the first three weeks after the fall of the petals in the case of the Bramley's Seedling, but in the Worcester Pearmain this rapid decrease lasts for four weeks. Starch synthesis then begins, and the concentration of residue either remains constant, as in Fig. 1, or rises slightly in the case of the Worcester Pearmain (see Table VIII). In Bramley's Seedlings the concentration starts to decrease again in the last fortnight of August, and continues to fall until the end of October, when starch hydrolysis is complete. The process is similar in the case of the Worcester Pearmain, but the fall in concentration occurs in the middle of July. No starch was detected in the early analyses of either variety of apple. Its synthesis begins about three weeks after petal fall in both cases, and the concentration increases from the middle of June to the end of July in the Worcester Pearmain, and to the end of August in the Bramley's Seedling; it then falls continuously until the end of October, when starch hydrolysis is complete. For Bramley's Seedling the maximum starch concentration reached at any time was 1.46 per cent., while for Worcester Pearmain it reached a value of 2.03 per cent.

The concentration of polysaccharides unhydrolysed by N/75 acid falls rapidly until the end of June, then more gradually, and finally reaches a constant value in September. The concentration of pectin also decreases, but a constant value is reached in the middle of June. Since the apple is still increasing in size, small amounts of these two substances must be continually formed in order to keep up the concentration.

The curve drawn through the values for the concentration of the alcohol-insoluble residue less the values for the starch is similar to those for the concentration of pectin and acid-insoluble residue. The concentrations of all constituents of the residue except the starch therefore fall off continuously during growth, the rate of fall diminishing as the fruit approaches maturity. The fluctuations in the residue curve must therefore be entirely due to the synthesis of starch in the growing fruit.

The values obtained for the hemicellulose fraction, calculated as the difference between the concentration of alcohol-insoluble residue and the sum of the starch, pectin, and material insoluble in N/75 acid, were plotted, and the curve through the points was found to have the same general form as that for the pectin, acid-insoluble residue, and alcohol-insoluble residue less the starch. This hemicellulose fraction is not hydrolysed again as the fruit ripens on the tree.

Figs. 2 and 3 show the time-quantity curves for the weight of starch in grammes per apple, for the two varieties of apple, Bramley's Seedling and Worcester Pearmain, during two consecutive growing seasons. For 1929 Bramley's Seedlings the maximum weight of starch per apple reached is 0.70 grm., but in 1930 a weight of 1.286 grm. is attained. For Worcester Pearmain the value is 0.798 in 1929 and 1.11 in 1930. A comparison of

the tables will show that the actual starch concentrations for either variety of apple during the two seasons are very similar on any particular date.

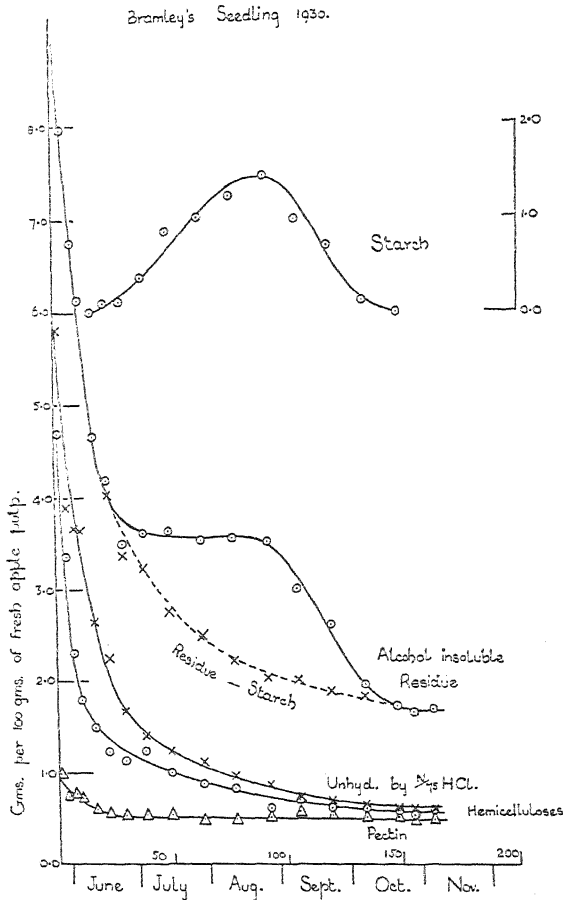


FIG. 1. Bramley's Seedling apples, 1930. Percentages in the fresh weight of alcohol-insoluble residue, starch, pectin, hemicelluloses, and residue insoluble in N/75 HCl during growth.

The variation in the values for the weight of starch per apple from year to year is due to the variation in the size of the fruits.

Corresponding curves for the weight of hemicelluloses in grammes per apple are given in Fig. 4. Unlike starch, these substances accumulate throughout the growing season and are not hydrolysed again to any extent as the fruit reaches maturity. For Bramley's Seedlings there is probably a small increase in the amount of these substances so long as the fruit is increasing in size, but the rate of accumulation during the last month is very small. For Worcester Pearmain a maximum value appears to be reached at the beginning of September. This hemicellulose fraction is

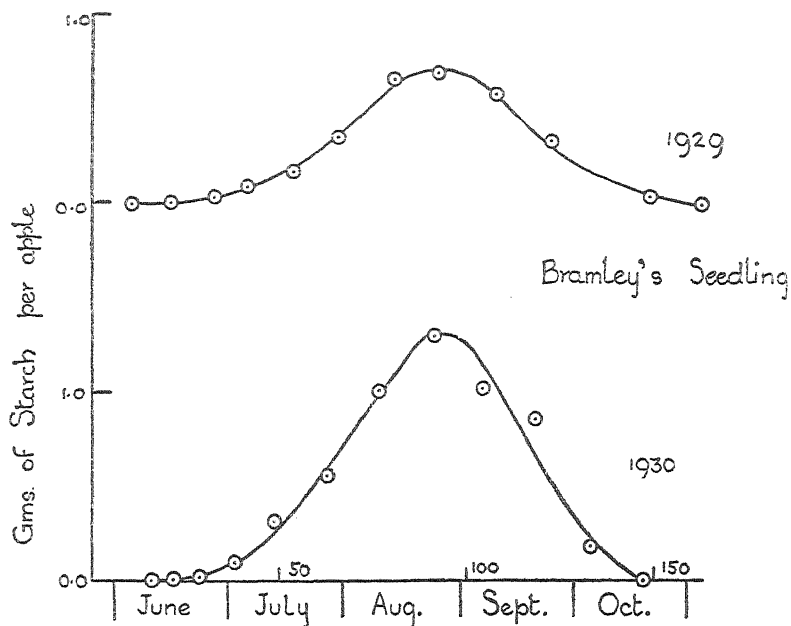


FIG. 2. Bramley's Seedling apples, 1929 and 1930. Amount of starch in grammes per apple during growth.

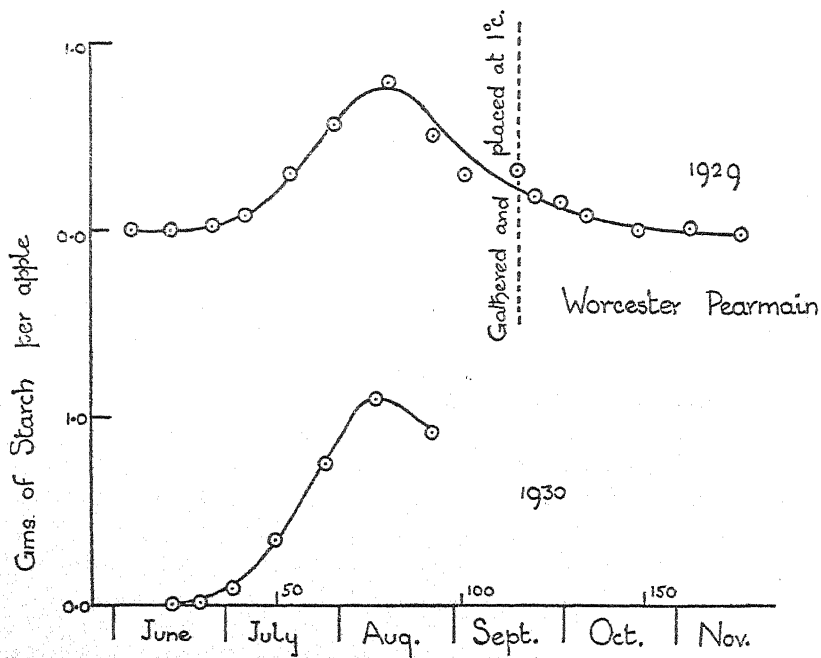


FIG. 3. Worcester Pearmain apples, 1929 and 1930. Amount of starch in grammes per apple during growth.



essentially a cell-wall constituent, and is closely related to pectin, and there appears to be no inter-relation between the hemicelluloses and the starch.

Since it is known (23) that in some varieties of apples there is a small

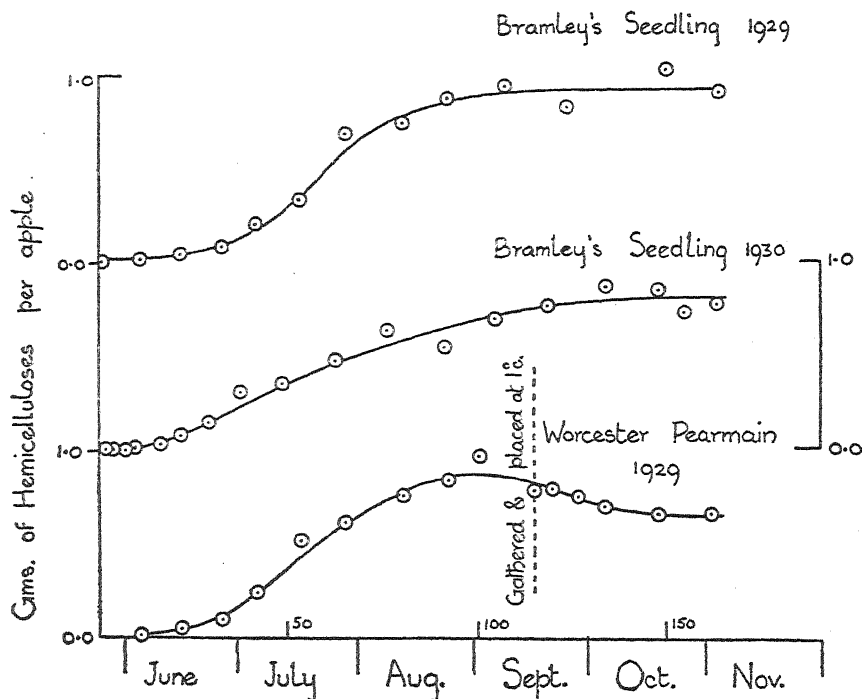


FIG. 4. Bramley's Seedling apples 1929 and 1930, and Worcester Pearmain apples, 1929. Amount of hemicelluloses in grammes per apple during growth.

loss of alcohol-insoluble residue during storage, an investigation has been carried out on the changes in concentration of pectin, residue unhydrolysed by N/75 acid, and hemicelluloses in Bramley's Seedling and Worcester Pearmain apples during storage at 1°C. The 1929 set of Worcester Pearmain were found to contain 0.6 per cent. of starch at the picking date, so the values for the starch and hemicelluloses for those apples during cold storage are included in Figs. 2-4.

Fig. 5 shows typical curves for the analysis of alcohol-insoluble residues from mature apples during storage. The results given are those for Bramley's Seedling 1929, which had been gathered about a fortnight before the normal picking date. They are expressed as percentage in original fresh weight of apple tissue.

It was found that the hemicellulose fraction decreases very slightly in amount, though at the end of five months in cold store 0.6 per cent. of the

original fresh weight, or more than 30 per cent. of the alcohol-insoluble residue, is still present in this form. Thus only 0.2 per cent. of this material is lost during storage at 1°, and it will be shown later that it is improbable

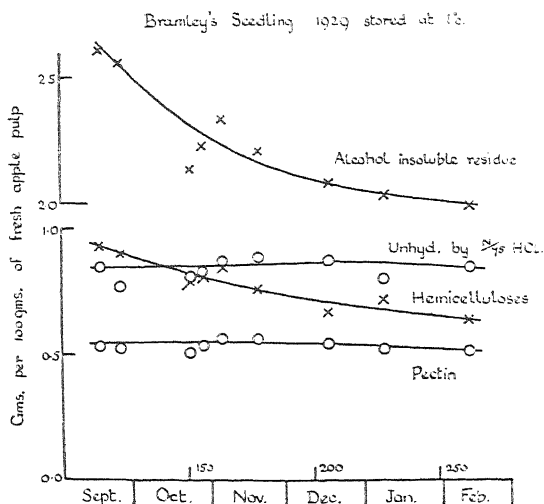


FIG. 5. Bramley's Seedling apples, 1929. Percentages in original fresh weight of alcohol-insoluble material, pectin, hemicelluloses, and residue unhydrolysed by N/75 HCl during storage at 1° C.

that this fraction serves as a reserve food supply for the fruit. This supports the view that only sugar and acid can be used by the plant for respiration.

The unhydrolysed fraction of the residue appears to increase very slightly in amount up to November, and then to remain constant, and it is possible that a portion of the hemicellulose fraction may change into a more stable form during the first month or so in cold store.

The amount of pectin remains constant throughout the storage period. This is in accordance with observations made by Carré (10), who found that the pectose and soluble pectin content of stored apples remained constant from the time of picking to the end of February.

These results indicate that when the starch has gone, loss from the alcohol-insoluble residue is accounted for by loss of hemicellulose.

#### IV. INVESTIGATION OF THE FRACTION OF ALCOHOL-INSOLUBLE APPLE RESIDUE OTHER THAN STARCH OR PECTIN WHICH IS EXTRACTED BY BOILING N/75 HCl.

It is now recognized that the carbohydrates of the cell-wall consist of quite large amounts of material which are not simple condensation products of aldose sugars. The recent work of Nanji, Paton, and Ling (33),

O'Dwyer (42), Candlin and Schryver (9), and Norman (37) establishes the fact that the cell-wall materials of plants contain complex molecules consisting of uronic acids in combination with hexose and pentose sugars. To this class of substances pectin belongs, and it is now becoming clear that other molecules of a similar nature exist which are possibly only distinguished from pectin by their lower uronic acid content, as suggested by Candlin and Schryver (9).

Such substances, which can be obtained from plant tissues by the action of alkalis of varying strengths, have been termed hemicelluloses, and more recently Candlin and Schryver have suggested the term polyuronides to describe substances consisting of sugars linked to a uronic acid. It seems likely that some substance of this class is present in apple tissue.

Many methods of extraction have been used to isolate hemicelluloses from plant tissues since Schulze (50) first showed that by treatment with alkali and precipitation with dilute acid a substance similar in character to cellulose could be obtained from plant tissue, to which he gave the name 'hemicellulose'. Hemicelluloses have since been isolated from various sources, including timbers (41, 42, 43), wheat bran (39), and apple wood (21), and have been shown to yield pentoses and hexoses when hydrolysed with acid. O'Dwyer (41, 42, 43) was the first to attempt a systematic study of these substances, and she used various timbers as the source of hemicelluloses. Her method consisted in the extraction of sawdust with cold 4 per cent. caustic soda after the removal of the water-soluble constituents, pectic substances, and protein. The alkaline extract was acidified with glacial acetic acid when hemicellulose A was obtained. Hemicelluloses B and C were precipitated by the subsequent addition of different amounts of alcohol or acetone to the acid solution. Hemicelluloses yield pentoses, hexoses and uronic acids on hydrolysis with acid. Other workers have followed O'Dwyer's method with slight variations, and similar results have been obtained by them. Norris and Preece (39) isolated four hemicelluloses from wheat bran, and Preece (46) has obtained the same number from maize cobs.

Cashmore (12) and Henderson (24) extracted the fibre of the flax plant with water as well as with alkali, and they found substances which are soluble in water in addition to the hemicelluloses which are extracted with cold caustic soda. Pentoses, hexoses, and uronic acids were identified among the hydrolysis products of the water-soluble fraction. Peterson and Hixon (45) state that alkaline extraction does not furnish a sharp distinction between cellulose and hemicellulose, and find that for the tissue of the cornstalk the results for alkaline extraction do not agree with those obtained by acid hydrolysis.

Gerhardt (21) has carried out a careful study of the effect of acid and alkaline hydrolysis on young apple wood, and his results are of considerable

interest. He finds that acid and alkaline hydrolysis remove about the same amount of material from the tissue but that their specific action on the tissue is different. By extraction of the wood with cold 4 per cent. NaOH, and addition of alcohol to the neutralized extract, a hemicellulose was obtained which yielded arabinose and xylose among its hydrolysis products.

Candlin and Schryver (9) divide the substances in the cell-walls of plants into three classes—lignins, hemicelluloses, and pectins. The hemicelluloses and pectins are formed by the conjugation of uronic acids with sugars, but pectins have a larger acid content than have hemicelluloses. These workers state, however, that pectins cannot be extracted with alkali or with water. This statement appears to be quite unjustifiable, since it is well known that soluble pectin may be washed out of plant tissues with cold water, and that boiling caustic soda solution (0.05 per cent.) will remove quantitatively the pectic acid and pectic substances of the middle lamella (7). Candlin and Schryver find that by treatment of pectin with weak alkali at room temperature they obtain among other products substances which resemble in all respects hemicelluloses isolated directly from timbers. Norman and Norris (38) were unable to repeat this experiment. They found, however, that if pectin was treated with such a weak oxidizing agent as Fenton's reagent, substances were obtained which bore a strong resemblance to the hemicelluloses. They suggest that this supports the view that hemicelluloses may be formed in nature by the protracted mild oxidation of pectin.

In a short review of the work on hemicelluloses up to 1929 which has been written by Murneek (32) the important part that substances of this type may play as storage carbohydrates in woody plants has been emphasized.

Hitherto, the only measure of hemicelluloses in the apple has been in terms of the reducing power produced by hydrolysis with 2.5 per cent. acid. Thus, Tottingham, Roberts, and Lepkovsky (54) included estimations of acid-hydrolysable material other than starch in their analyses of apple wood. After removal of starch by saliva, the residue was boiled with HCl and the sugar in the solution estimated by the Defren-O'Sullivan (15) method. The reducing power calculated as glucose was between 20 per cent. and 30 per cent. of the dry weight. Gerhardt (20) and Murneek (30), (31) have applied the same method of estimation to the fruit of the apple. After removal of the dextrin and the starch from the alcohol-insoluble apple residue, the material was boiled with 2.5 per cent. acid and the filtrate neutralized, cleared with lead acetate and sodium carbonate, and the sugar in the cleared solution estimated. Gerhardt (20) has used apples gathered at different dates, and after different lengths of time in cold storage, and he has found amounts of acid hydrolysable material calculated as glucose

varying from 0.2 to 1.5 per cent. of the fresh weight. Murneek (31) has worked on the immature fruit and has found as much as 8.0 per cent. of the fresh weight to consist of easily hydrolysable material at a very early stage of development.

This acid hydrolysis method has very obvious disadvantages. First, it is clear from the work of O'Dwyer (42) that the reducing power gives no measure of the amount of hemicellulose present, since hemicellulose may contain varying amounts of uronic acids, and in one case quoted by her, a sample of hemicellulose yielded as much as 63 per cent. of galacturonic acid. Secondly, with acid hydrolysis pectin will contribute some reducing power to that of the hydrolysate. Results which are given for hemicellulose must therefore include the hydrolysis products of pectin. Thirdly, clarification of solutions containing HCl or  $\text{H}_2\text{SO}_4$  with lead acetate offers serious difficulty owing to the precipitation of lead chloride or lead sulphate. It has been calculated that 100 c.c. of 2.5 per cent. HCl would give more than 9 grammes of lead chloride on the addition of excess lead acetate. If the solution is made up to volume before filtering, this large precipitate would introduce an error into any values which are obtained for the cleared solution.

In consideration of the disadvantages of acid hydrolysis it was thought that an investigation of the nature of substances other than starch or pectin which are extracted by dilute acid from apple tissue should include an attempt to isolate such substances by the different methods of extraction noted above. This attempt has been made, and, further, the properties of the substances isolated have been examined with a view to their identification.

For the purpose of these investigations alcohol-insoluble apple residue containing no starch was used, since analyses of the apple at different stages of growth has shown that the easily hydrolysable fraction of the tissue is present at all stages of development.

About 0.5 gram. of residue was boiled under a reflux condenser with 100 c.c. N/75 HCl for 3 hours, filtered, and the filtrate neutralized and diluted to 250 c.c. A brown solution was obtained which possessed a reducing power equivalent to 14 per cent. of glucose, estimated by oxidation with alkaline ferricyanide or alkaline iodine. Since it is known that colouring matter may affect the ferricyanide and iodine oxidations (5, 55), the reducing power of the solution was again determined after decolorizing by boiling with 'Suchar'. A value of 11 per cent. of glucose was obtained.

The degradation products of pectin are probably responsible for part of the reducing power obtained after boiling apple residue with N/75 acid. In order to obtain a measure of the reducing power due to pectin, 1.5 gram. apple residue containing no starch were boiled with N/75 HCl for 3 hours,

and the portion insoluble in acid was filtered off. The filtrate was neutralized, decolorized by boiling with 'Suchar', and diluted to 250 c.c. The pectin was precipitated as calcium pectate from duplicate 75 c.c. samples as described before, and the weight of calcium pectate determined. The filtrates and washings were neutralized, evaporated down to small bulk, and made up to 250 c.c., and the reducing power of 5 c.c. determined by oxidation with hypiodite. (The method of oxidation with alkaline ferricyanide cannot be used owing to the presence of calcium in the solution. A precipitate of calcium carbonate is formed on the addition of the ferri-cyanide-sodium carbonate solution.)

Seventy-five c.c. of the original solution, after boiling with acid and decolorizing with 'Suchar', were diluted to 250 c.c. without precipitating the pectin, and the reducing power determined. A 'blank' determination was carried out, using the same reagents, but this showed no apparent reducing power, though the presence of the calcium chloride rendered the endpoint a little less sharp than in the case of an ordinary sugar solution. The results obtained were calculated as apparent percentage of glucose in the residue before and after precipitation of the pectin. They are shown in Table X and indicate that only about 25 per cent. of the reducing power can be due to the hydrolysis products of pectin.

TABLE X.

*Reducing Power of Extract obtained by boiling Apple Residue with  
N/75 HCl estimated before and after precipitating Pectin.*

	Percentage reducing power calculated as glucose.
Before precipitating pectin . . .	13.37
After " " " " " " " " " " " "	10.07
Reducing power due to pectin . . .	3.30
Percentage pectin in residue (as calcium pectate) . . .	18.60

It is thus clear that alcohol-insoluble apple residue contains substances other than starch or pectin which possess a reducing power or which are hydrolysed by very dilute acid to reducing substances.

Preliminary experiments showed that far more material was extracted from alcohol-insoluble apple residue containing no starch by the action of boiling N/75 acid, boiling water, and cold 4 per cent. alkali than could be accounted for by pectin. Parallel extractions of apple residue with N/75 acid, water, and alkali were accordingly carried out on a larger scale with a view to the isolation of the substance or substances other than starch or pectin which are extracted by these solvents. Sulphuric acid was used instead of hydrochloric acid, for acid hydrolysis, and this was subsequently removed from the solution by barium hydroxide. A water extraction was

carried out, since it was thought that this would involve less chance of hydrolysis of the substances during the extraction process; and since a method of alkaline extraction has been used extensively for the isolation of hemicelluloses, the effect of alkaline treatment on apple residue has been investigated, in order to discover whether any substance could be isolated by these means, other than those obtained when water or dilute acid was used as the extracting agent.

#### *Acid extraction.*

For the acid extraction 20 grm. of alcohol-insoluble apple residue were boiled under a reflux condenser with 1,500 c.c. N/75  $\text{H}_2\text{SO}_4$  for 3 hours, filtered, and the extraction of the residue by acid was repeated twice more. The insoluble fraction was washed with hot water, dried at  $100^\circ$ , and weighed. The united filtrates were neutralized with baryta, which precipitated some of the pectin with the sulphate. A separate determination of pectin was carried out on another portion of the same sample of residue after hydrolysis with N/75  $\text{HCl}$ .

The precipitate was filtered off and the filtrates were boiled down to 500 c.c. An excess of lime-water was added to precipitate any pectin which had not been removed by the baryta (40). The solution was allowed to stand overnight, filtered, and carbon dioxide was passed into the filtrate to remove excess calcium. After removal of the calcium carbonate by filtration the solution was evaporated down to 500 c.c. and twice its volume of 95 per cent. alcohol was added. A white flocculent precipitate was formed, which was filtered off after 24 hours, dried *in vacuo* over  $\text{H}_2\text{SO}_4$  and then at  $100^\circ$ , and weighed. (Fraction I.)

The filtrate from (I) was evaporated down on the water bath to a syrup and absolute alcohol was added. A further precipitate was formed which was filtered off in a Gooch crucible, dried *in vacuo* and weighed. (Fraction III.) A small amount of brown sticky material still remained soluble in absolute alcohol. This was also dried *in vacuo* and weighed.

#### *Water extraction.*

A similar extraction was carried out by extracting 20 grm. of alcohol-insoluble residue three times with boiling water. After filtering off the insoluble fraction, which was dried and weighed, the filtrates were boiled down to 500 c.c. and an excess of lime-water was added to precipitate the pectin (40). The calcium pectate was filtered off after 24 hours, and washed by repeatedly boiling with water until the washings were free from calcium. It was then dried at  $100^\circ$  and weighed. Carbon dioxide was passed into the filtrates and the subsequent procedure was similar to that already described for acid extraction. Two fractions were obtained as

before, one of which was insoluble in 60 per cent. alcohol (II), and the other of which was insoluble in absolute alcohol (IV).

The results of the analyses showed that these fractions accounted for about 98 per cent. of the residue by both methods of extraction. They are shown in Table XI.

TABLE XI.

*Extraction of Apple Residue with N/75 H<sub>2</sub>SO<sub>4</sub> and with Water.*

	% of residue.	
	Extracted with N/75 H <sub>2</sub> SO <sub>4</sub> .	Extracted with water.
Insoluble in extracting agent . . .	45.65	61.15
Pectin (weighed as calcium pectate) .	28.36	21.70
Insoluble in 60 % alcohol (not pectin) .	5.85 (I)	2.10 (II)
Insoluble in absolute alcohol (not pectin)	14.95 (III)	11.25 (IV)
Apparently soluble in absolute alcohol .	3.55	1.75
Total amount recovered	98.36	97.95

The four fractions obtained (I, II, III, IV) were yellowish powders readily soluble in cold water. On the addition of calcium chloride to their solutions there was no precipitate of calcium pectate. They gave no colour with iodine (cf. 39, 46).

Fractions I, II, and IV were found to have no action on Fehling's solution, but Fraction III, extracted from the residue by boiling with N/75 H<sub>2</sub>SO<sub>4</sub> and insoluble in absolute alcohol, possessed a strong reducing power. This fraction charred at 100° while the other three samples were unchanged by this treatment. The presence of this substance in the solution after dilute acid extraction would account for the reducing power of the solution noted on p. 620.

The uronic anhydride content of all four fractions was estimated by Nanji, Paton, and Ling's (33) method, adapted for small quantities by a micro method devised by Buston (8). The ash content of each was also determined. The results are shown in Table XII.

TABLE XII.

*Uronic Anhydride and Ash Contents of Hemicellulose from Apple Tissue.*

		% Uronic Anhydride.	% Ash.
Ext. by N/75 H <sub>2</sub> SO <sub>4</sub> insol. in 60 % alc.	I.	17.87	1.37
Ext. by N/75 H <sub>2</sub> SO <sub>4</sub> insol. in absol. alc.	III.	6.87	2.03
Ext. by water insol. in 60% .	II.	18.40	2.30
Ext. by water insol. in absol. alc.	IV.	None	1.64

On hydrolysis with dilute acid, substances having a strong reducing action on Fehling's solution were produced in every case. Osazones



prepared from the hydrolysis products all proved to be arabinosazone. There was no indication of the presence of any other sugar. The osazones after recrystallization gave melting points of 156–160°, and the presence of arabinose was further confirmed in Fractions III and IV by the preparation of the diphenylhydrazones (35). After recrystallization from 60 per cent. alcohol these gave melting points of 206°.

Owing to lack of material, this test could not be applied to Fractions I or II.

The properties and reactions of these four fractions thus indicate that the substances extracted by dilute acid and by water from the tissue and precipitated by 60 per cent. alcohol are identical. The differences in the uronic anhydride content of these two fractions lies within the limits of experimental error of the method used for the determination. The substance belongs to the class of compounds termed by Candlin and Schryver 'polyuronides', that is, it consists of a uronic anhydride linked to a pentose sugar. The simplest type of molecule that would account for the properties of this substance consists of six anhydro-arabinose groups linked to one uronic anhydride unit. Such a compound, or its polymer would yield 18.6 per cent. uronic anhydride on hydrolysis with acid.

Fraction IV, obtained from the water extract by precipitation with absolute alcohol, is a true polysaccharide, since it contains no uronic groups. It is, therefore, quite distinct from the 'polyuronic' already discussed, and its separation from that fraction must have been complete.

Fraction III, obtained from the acid extract by precipitation with absolute alcohol, probably consists of a mixture of substances. Since charring occurs on heating there is probably some free sugar present, and it is suggested that the process of boiling with the dilute acid has hydrolysed a portion of the 'polyuronic' fraction (which is normally insoluble in 60 per cent. alcohol) into its two component parts—a sugar fraction, which causes the strong reducing action on Fehling's solution, and a substance of a uronic acid nature. These two hydrolysis products are soluble in 60 per cent. alcohol, but are insoluble in absolute alcohol.

The polysaccharide found in Fraction IV must be also present here, and this may or may not have become partially hydrolysed by the action of the acid.

#### *Alkaline extraction.*

For the alkaline extraction 10 grm. of alcohol-insoluble residue were extracted in the cold by 300 c.c. 4 per cent. caustic soda for 24 hours. The residue was filtered off through glass wool, washed with large quantities of cold water till the washings were no longer alkaline to litmus, dried at 100°, and weighed. Norris's and Preece's (39) method for the precipitation of hemicelluloses was then followed. The filtrate and the washings were neutralized

with glacial acetic acid and the pectic compounds were precipitated, as already described, by the addition of lime-water, dried and weighed. The filtrate, after removal of excess calcium by  $\text{CO}_2$  was boiled down to 100 c.c. An excess of glacial acetic acid was then added, but no precipitate corresponding to O'Dwyer's hemicellulose A was formed, nor was there any precipitate of hemicellulose B after the addition of half the volume of acetone to the solution. When twice the volume of acetone had been added, however, a white precipitate came down which became granular when the mixture was heated on the water bath. This was filtered off through a weighed Gooch crucible, dried *in vacuo* over  $\text{H}_2\text{SO}_4$  and weighed. This fraction amounted to 5.2 per cent. of the original weight of the residue. The substance was a white powder, readily soluble in cold water, which gave no colour with iodine and which had no reducing action on Fehling's solution. It was found to have an ash content of 1.67 per cent. and to contain no uronic acid groups. Hydrolysis with acid produced reducing substances. The osazone was prepared after boiling with acid as before, and arabinose was the only sugar which could be detected. After recrystallization the osazone gave a melting point of  $159^\circ$ . The presence of arabinose was confirmed by preparing the diphenylhydrazone (35). After recrystallization this gave a melting point of  $204^\circ$ . The substance thus appears to be a true polysaccharide.

The substance isolated was thus similar in properties to that obtained by extraction of the apple residue with water and insoluble in absolute alcohol. No trace of the polyuronide fraction could be detected by this method of extraction. It appears, therefore, that cold 4 per cent. caustic soda solution does not extract a polyuronide from apple residue. This point was further demonstrated by the fact that the polyuronide extracted by boiling water or by N/75 acid from apple tissue and precipitated by 60 per cent. alcohol was almost insoluble in cold 4 per cent. caustic soda solution, though it was readily soluble in water. A portion of this substance which had been treated with alkali was found to be no longer soluble in water, and, further, when strong caustic soda solution was added to a water solution of the substance it was precipitated as the sodium salt. The alkali-treated hemicellulose was readily soluble in dilute acid.

The properties of the two fractions made it clear that two different substances had been isolated from apple tissue by the action of water alone. One is a polyuronide and the other a true polysaccharide. These observations confirm those made by Anderson (1), who has suggested that there are two distinct classes of hemicelluloses—acid hemicelluloses and polysaccharide hemicelluloses—both of which may be present in plant materials.

Nanji, Paton, and Ling (33) found that pectinogen, obtained by the method of alcohol precipitation, is always contaminated with two substances, one of which yields  $\text{CO}_2$  when boiled with acid and is not precipitated by

calcium chloride, while the other is of carbohydrate nature. This also is in agreement with the present observations.

In addition, both Cashmore (12) and Henderson (24) obtained substances, which they called hemicelluloses, by the action of water under pressure on the flax plant. Cashmore was able to identify two substances in his hemicellulose fraction, one of which was insoluble in 70 per cent. alcohol and gave 20.8 per cent. uronic anhydride when hydrolysed with acid. The other was soluble in 70 per cent. alcohol and contained no uronic anhydride. These substances are therefore very similar in properties to those obtained from apple tissue by water extraction.

According to Schorger (49), Candlin and Schryver (9), Peterson and Hixon (45), and Anderson (1) hemicelluloses are insoluble in water but soluble in dilute alkali. The water-soluble substances which have been isolated from apple tissue show all the other properties generally attributed to hemicelluloses, and it appears therefore that insolubility in water is not a good criterion of a hemicellulose.

The hemicelluloses obtained by O'Dwyer (41, 42, 43), Gerhardt (21), Norris and Preece (39), and Preece (46) by alkaline extraction must necessarily be different from either of those here isolated from apple tissue, since those workers employ a preliminary treatment with ammonium oxalate to remove the pectin, and this would presumably remove both hemicelluloses which have been identified in apple residue.

The action of taka-diastase on the two substances isolated after the boiling-water extraction was investigated. Weighed quantities of each were incubated with taka-diastase at 38° for 24 hours, the solutions cleared and made up to volume in the usual way. No differences were observed in the ferricyanide titrations between the values for these solutions and the value for a taka-diastase blank. This enzyme, therefore, does not act upon these substances to give reducing sugars. A paper has recently been published by Preece (47) on the hemicelluloses of boxwood. This writer finds that boxwood contains a small amount of material soluble in hot water, in addition to free and combined hemicelluloses obtained by alkaline extraction. The 'free hemicellulose' was soluble in hot water, and no simple sugars could be detected after hydrolysis of the substance with taka-diastase. Since the amounts of these substances isolated after extraction of the alcohol-insoluble apple residue with boiling water, together with pectin and residue insoluble in water, accounts for 98 per cent. of the original residue, it appears certain that in the starch estimations no error is introduced by the presence of a water-soluble material other than starch extracted during the gelatinization process, which is acted upon by taka-diastase to give reducing sugars. Thus the use of taka-diastase for the determination of starch in apple tissue is further justified.

## V. DISCUSSION.

Quantitative estimations of starch in Bramley's Seedling and Worcester Pearmain apples have been carried out during two growing seasons, and, in addition, determinations of pectin have been made on the same samples.

The results obtained have made it clear that apple tissue contains considerable quantities of readily hydrolysable material, other than starch or pectin, which has been shown to belong to the class of substances known as hemicelluloses. This fraction is extracted from alcohol-insoluble apple residue by boiling with N/75 acid, and it has been shown that it will entirely account for the fraction of the residue other than starch or pectin which is extracted by acid of this strength.

There appears to be no substantial evidence that the hemicelluloses present are a source of reserve carbohydrate in the apple, and the results obtained during the present investigation indicate that they do not disappear during the storage life of the apple, and the sugar produced on hydrolysis is not of the type likely to be used in respiration.

Murneck (31) found that for developing apples a high concentration of total sugar is coincident with a low 'acid hydrolysable' or hemicellulose content at the time when the flowers are fully open. He suggests that this indicates that large amounts of hemicellulose have been converted into sugars during the period of blossoming. Immediately after petal fall the sugar is apparently recondensed to hemicellulose, since its concentration decreases. Howlett (26) has analysed the flowers and fruit of three varieties of apples at a very early stage in development, and he also finds an increase in concentration of total sugars up to the time of full opening and a subsequent decrease when the petals fall. No significant changes in the concentration of acid hydrolysable material, either in the flowers up to full bloom or in the young fruits subsequent to petal fall, are noted by him. Howlett suggests that the loss in soluble carbohydrate may be accounted for by an increased respiration or by a 'backward translocation into the cluster bases', or, less likely, by a change to a type of carbohydrate not estimated by the methods of analysis used.

Howlett observed further, however, that at full bloom the petals contain two-thirds of the total reducing sugars of the entire flower, although they only comprise one-third of its fresh weight. If this is so, the increase in sugar concentration shown by the analysis of the entire flower must be largely due to the accumulation of sugar in the developing petals. Howlett found that the sugar concentration reached a maximum at full bloom and decreased again as the petals fell, and it seems almost certain that the carbohydrate loss may be entirely accounted for by the dropping of the petals.

The present sets of analyses were begun at the stage when the petals

had just fallen, so no confirmation of the rise and fall of sugar concentration and the simultaneous fall and rise of hemicellulose content prior to this time, as recorded by Murneek, could be obtained. The subsequent course of the concentration curves for both sugar and hemicelluloses is similar to that given by him. The concentration of total sugars continues to increase steadily (see Archbold 4), while the hemicellulose concentration falls rapidly at first, then more gradually, and finally reaches a constant value. The values for the weight of hemicelluloses as grammes per apple show that these substances accumulate in the fruit from the time of petal fall up to a short while before the normal picking date. From then the values remain fairly constant, or fall off very slightly, and there is no evidence that these substances are hydrolysed by the apple to a soluble form of carbohydrate. McKinnis (29) has shown that apples contain no free pentose. The fact that arabinose is the only sugar identified on hydrolysis of either hemicellulose with 2.5 per cent. acid suggests that these substances are not likely to serve as an important source of reserve carbohydrate for the plant.

Magness (28), in the course of investigations on the chemical constitution of Bartlett pears, has found that there is a considerable decrease of acid-hydrolysable material other than starch as the fruit ripens on the tree, and he concludes that as the fruit develops much material other than starch is converted into sugar. Since, however, his results are expressed in terms of concentration, the absolute amounts of hemicellulose would probably show an increase during ripening.

In common with other hemicelluloses (see 9, 17, 32, 42), those of the apple are much more closely allied to pectin both in constitution, and presumably in function, than to starch or to true cellulose.

Thaysen and Bunker (51) divide the hemicelluloses into two natural groups, those which act as reserve food materials for the plant, and those which serve as structural support for the cell-walls and which yield pentoses on hydrolysis with acid. It would appear from the present investigation that all hemicelluloses are structural, and that they only differ from one another in the degree of complexity of their molecules.

Norman (37) has demonstrated the similarity between hemicelluloses and gums, and he suggests that in nature both hemicelluloses and gums are produced by the partial decarboxylation of pectin. This view is supported by Norman and Norris (38), who have obtained substances which bear a strong resemblance to hemicelluloses by the mild oxidation of pectin.

O'Dwyer (42) and Candlin and Schryver (9) state that lignified tissues contain large amounts of hemicelluloses, with only traces of pectin, while non-lignified tissues contain relatively large amounts of pectin and small amounts of hemicelluloses. These workers suggest that this bears out the

view that decarboxylation of the pectin takes place during lignification. Onslow (44) considers that early lignification takes place in tissues destined to become xylem, while in fleshy, unligified tissues pectic substances are accumulated. Thus all cell-walls contain cellulose and hemicellulose, and in unligified walls the pectin complex is developed.

The present work has shown that in the unligified tissue of apple the concentration of hemicelluloses is higher than that of pectin at all stages of development of the fruit, and in the very early stages the amount of hemicelluloses present may be four times the amount of pectin. In the growing fruit the accumulation of these two substances has proceeded simultaneously, and there is no evidence that pectin has been converted into hemicellulose.

Carré (10) has shown that when apples reach a stage of senescence, after seven months in cold store, there is a disappearance of total pectic constituents. This, she suggests, is due to a process of decomposition, first to pectic acid, and then further to galactose, arabinose, and methyl pentose. It is possible that when the fruit becomes over-ripe the pectin is converted into compounds of a hemicellulose nature by a process of partial decarboxylation.

This point requires further investigation.

## VI. SUMMARY.

A method has been developed for the determination of starch in apple tissue. After a preliminary extraction of the alcohol-insoluble apple residue with cold potassium oxalate solution to remove some of the pectin, the starch is hydrolysed by means of taka-diastase, and the glucose and maltose in the hydrolysis mixture are estimated by oxidation with alkaline ferricyanide and alkaline iodine.

Determinations of starch have been carried out on alcohol-insoluble material obtained from samples of Bramley's Seedling and Worcester Pearmain apples. Samples were collected at intervals of a few days, starting with the flowers immediately after the fall of the petals. The changes were followed up to the stage of the mature fruit, and later in storage. Starch begins to appear in the middle of June, rises to a maximum concentration of 1.5–2 per cent. of the fresh weight at the end of July in the Worcester Pearmain and at the end of August in the Bramley's Seedling, then falls again, disappearing completely at the end of October in both varieties of apple.

Estimations of pectin were carried out on the same samples by extracting the tissue with N/75 HCl. The acid was found to remove much more material from the residue than could be accounted for by starch and pectin, and it is concluded that apple tissue contains some readily hydrolysable polysaccharide other than starch or pectin.

Two water-soluble substances have been isolated from apple residue which are not hydrolysed by taka-diastase nor precipitated by calcium chloride. One is shown to be a polyuronide and the other to be a polysaccharide. Both yield arabinose on hydrolysis and belong to the class of substances known as hemicelluloses.

Both hemicelluloses and pectin in the developing apple increase steadily to a constant value which does not fall to any extent during storage. This observation, together with a consideration of the hydrolysis products of the hemicelluloses, suggests that they do not serve as a reserve carbohydrate supply for the fruit, but that in structure and function they are intimately related to pectin.

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# The Development and Cytology of the Leaves of Healthy and 'Silvered' Victoria Plum-trees.

BY

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With Plates XIX and XX and three Figures in the Text.

## INTRODUCTION.

THE work, the results of which are given in the following paper, originated as a study of the cytological phenomena in the 'silvered' leaves of Victoria plum-trees. The previous work on this subject has shown that, probably, the cytology of these diseased leaves is not normal, but the observations recorded are scattered and have not been correlated at all with the developmental stages through which the leaf passes. The importance of such a correlation in a rapidly growing organ like the leaf is self-evident if any conclusions are to be drawn from the observations. The problem, therefore, involved a comparative study of both normal and diseased Victoria plum-leaves at all stages of development throughout the year.

As far as the author is aware, there is no complete account of the stages of development through which a leaf passes before reaching maturity, although there is abundant literature on the mature structure of leaves. A study of the stages by which they attain their mature structure should be of interest from several points of view:—(1) It might shed light on the extent to which the structure of leaves is bound up with environment and the nature of the influence of environment on growing organs. (2) It might also be of use in determining the way in which foliage leaves have been evolved in the plant kingdom. (3) Finally, it is a matter of interest to know, as far as possible, the different developmental stories of these important plant organs which show such a wide range in their structure.

The development of the normal plum-leaf and other leaves worked out for the sake of comparison brought several interesting facts to light. It is hoped, therefore, in the future, to publish the results of work covering the wide range of leaf structure found in the higher groups of the plant kingdom,

but the results given in this paper will be confined to normal and 'silvered' *Victoria* plum leaves.

#### *Methods.*

Leaves of the *Victoria* plum were gathered at frequent intervals from February until November 1929. Five different fixatives were used to give several comparable series at every stage. The fixatives employed were Benda's fluid, Bouin's picric acid fixative, Gilson's corrosive sublimate fixative, Carnoy's fluid, and formalin acetic alcohol. The material was found to be difficult to fix and to embed so as to give satisfactory preparations, but after a large amount of preliminary work good preparations were obtained. Cedarwood oil was found to be preferable to xylol as a clearing reagent, and it was used exclusively throughout this investigation. Fresh material was also used, as far as possible, to check the general observations noted in the microtomed preparations. It was found to be impossible, however, to distinguish minute cytological details in the living nuclei.

A large variety of stains were used for the fixed material. The staining capacity of the protoplast varied considerably at the different stages of development, and some stains which were very useful in the later stages were absorbed so strongly in the meristematic stages that little differentiation was possible. The most generally used stains were brazilin (0.5 per cent. solution in 70 per cent. alcohol), and eosin and toluidine blue (Mann's method). Heidenhain's haematoxylin, gentian violet, and orange G., and alizarin and crystal violet were also used.

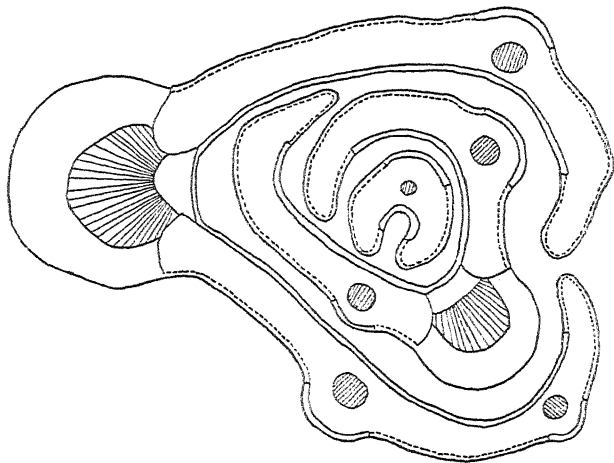
After the material had been embedded in wax with a melting point of 56° C., sections were cut from 5 to 8  $\mu$  in thickness.

#### *The Development and Cytology of Healthy Victoria Plum Leaves.*

A developing bud of a healthy tree in February consists of a large number of leaves folded up one inside the other. They are arranged in such a way that serial sections through the bud show a number of V-shaped sections of leaves which fit inside each other with the main vascular bundle forming the base of the V.

Sections through a bud that is just swelling so that the tips of green leaves protrude from its apex show that the mesophyll of the leaf consists of meristematic cells which are uniform in appearance and give no indication of the future layers which characterize the mature leaf. The outer leaf of such a bud perhaps shows the first signs of the characteristic shape of the palisade cells, but, at this early stage, this layer consists of cells only slightly elongated at right angles to the upper epidermis; they still possess large nuclei and dense cell contents.

One very noticeable feature about the young leaves is the difference in the vacuolation of the cells forming the upper and lower epidermis. The cells of the lower epidermis just below the future main vascular bundle are



TEXT-FIG. 1. Transverse section of leaves of an opening *Victoria* plum bud showing the extent of the vacuolation of the upper and lower epidermis. The continuous line within outline of leaf indicates limit of vacuolated epidermal cells. The broken line within outline of leaf indicates limit of meristematic epidermal cells. The area occupied by vascular tissue is hatched.

the first cells to vacuolate, but this occurs before any vascular elements are distinguishable. With the development of the xylem, the upper epidermal cells begin to vacuolate and vacuolation then spreads comparatively rapidly in this layer on either side of the main vascular bundle, so that the extent of the vacuolated epidermal cells is always slightly in advance of the development of the vascular system (see Text-fig. 1). Vacuolation does not proceed in the cells of the lower epidermis in this way, and, for some considerable time, the only vacuolated cells in this layer are those facing the vascular bundles. The lower epidermal cells between the vascular bundles remain meristematic long after the upper epidermal cells have become vacuolated, and they continue to divide by means of walls laid down at right angles to the leaf surface. The number of cells in the lower epidermis over a given length of the section is therefore greater than that in the upper epidermis.

The cells of the mesophyll remain undifferentiated until the leaf is of some considerable size and is beginning to flatten out. The shape of the cells, however, changes gradually and all become slightly elongated at right angles to the epidermis. This early elongation of all the cells of the mesophyll may perhaps be due to the effect of increasing concentrations of light on the leaves as they become separated from each other by the growth of the stem on which they are borne. Plum leaves, at this early stage

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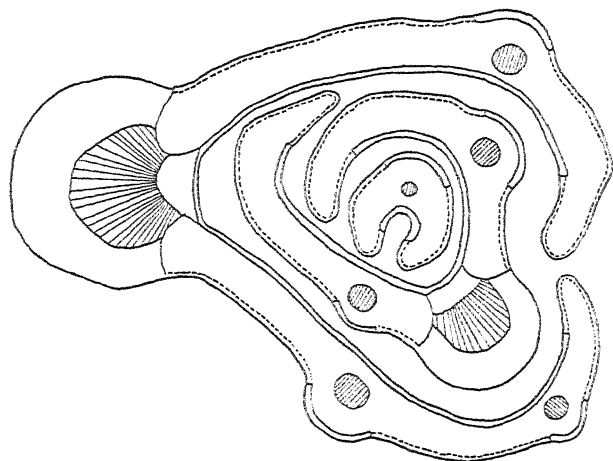
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before the leaf has flattened out, are very thin, and the intensity of light on all the mesophyll cells is probably of about the same magnitude. Cell division in the palisade layers goes on very rapidly in these young leaves, so that the cells come to be packed close together. They are narrow, brick-shaped cells because the new walls are formed at right angles to the epidermis; they contain large nuclei and heavily staining contents. This method of cell division and the consequent mutual pressure exerted by these cells on each other (owing to the presence of the vacuolated upper epidermal cells above them and the comparatively differentiated vascular bundles on either side) would account for the invariable shape of the palisade cells of such dorsiventral leaves. Some workers, however, consider that light intensity is an important factor in determining the shape of the palisade cells. Nuclear divisions also take place in the cells of the future spongy mesophyll, but in these layers the new wall is laid down in any plane of the leaf. During this rapid increase in the number of the cells of the mesophyll, the vacuolation of the individual cells has been going on very gradually and the chloroplasts begin to be distinguishable at the edge of the developing vacuoles. No intercellular space system and no stomata are present in these early stages.

The next stage observable in the development of the leaf is that of the stretching of the upper epidermal cells in a direction parallel to the surface. It is, at first, accompanied by the continued division of the palisade cells, and later by a similar but comparatively slight phase of extension in this layer too. The upper epidermal cells change their shape rapidly and become square in section, and finally elongate in the direction parallel to the surface of the leaf. At this stage, the leaf flattens out and the shiny yellow green upper surface is replaced by a noticeably duller surface, after which the leaf assumes its typical vivid but darker green. The extension of the leaf in area still continues, as this stretching of the upper epidermal cells goes on for some considerable time. The activity in the mesophyll cells takes place throughout the whole of the leaf, and no special part of the leaf was found to be more meristematic than any other part. New cells are continually being added to the length of the leaf as well as to the breadth.

The frequency of the division in the cells forming the spongy mesophyll evidently lags behind that of the palisade layers, so that, during this period of gradual increase in area of the leaf, the intercellular space system comes to be developed. The first intercellular space which is distinguishable is the sub-stomatal cavity, which becomes enlarged by the continued division of the cells of the lower epidermis and those of the mesophyll surrounding the cavity. One of these cells may occasionally break down, thus enlarging the space to a considerable extent. The formation of the rest of the intercellular space system takes place rapidly during the period of maximum



extension. At this stage, readjustment of the cells of the spongy mesophyll is taking place: the cells are vacuolating and enlarging and become separated from each other and so give rise to the typical intercellular



TEXT-FIG. 2. Stages in the formation of stomata in the leaves of *Victoria plum*.  $\times 350$ . *a*. Mother-cell of guard-cell, and sub-stomatal cavity. *b*. Showing the heavily stained middle lamella between the guard-cells and the continuous epidermis. *c*. A mature stoma with remains of the middle lamella substance still present.

spaces of this region. The spongy mesophyll cells still remain meristematic in the early stretching period, and divisions take place to a certain extent. The decrease in the rate of these divisions in the cells of the spongy mesophyll when the cell-walls of the leaf as a whole are passing through the phase of extension, is an important factor in the formation of the intercellular space system of the leaf.

The development of the guard-cells of the stomata takes place after the appearance of the sub-stomatal cavities. There is, at first, very little to distinguish the mother-cells of the guard-cells from the meristematic lower epidermal cells. They contain very large nuclei and dense protoplasm and soon begin to enlarge in a radial direction. The nucleus divides and the new wall is laid down at right angles to the surface of the leaf. The formation of the subsidiary cells follows after that of the guard cells; they are cut off after the division of the cells of either side of the guard-cells. Later, the wall between the two guard-cells begins to stain heavily with toluidine blue or brazilin. It is breaking down gradually and, at one stage in its dissolution, it takes the form of a dark band of stained material shaped like an hour-glass (Text-fig. 2, *b*). The cuticle is certainly continuous above the stomata at this stage and remains so until the lower epidermal cells have vacuolated and stretched.

While the middle lamella is dissolving, the inner walls of the guard-cells are thickening, and the latter gradually assume their characteristic mature shape. The mature stomata often come to be arched slightly above the general level of the epidermis. This is due to the continued divisions of the lower epidermis on either side of the sub-stomatal cavity. When the middle lamella between the guard-cells has disappeared, a flap of cuticle is left projecting from the outer surface of the walls of the guard-cells. After the stretching of the walls of the lower epidermal cells has taken place, the guard-cells become separated and the stomata then function for gaseous diffusion.

Ziegenspeck (14) found that in the growth of roots, the cells of the

extending zone went through a stage in which the cell-walls gave a blue reaction with iodine without any previous treatment. He identified this reaction with the stage of maximum extensibility of the wall. The cell-walls in these young leaves are, however, so thin and transparent that it was impossible to be certain what colour they gave with iodine, but it was certain that they all passed through a well-marked stage of extension. This noticeable increase in area of the cell-wall occurs simultaneously with the vacuolation of the protoplast, the final stages of which produce the structure of the mature leaf. This consists of one or two layers of palisade cell with numerous air spaces between the long walls of the individual cells and a spongy mesophyll layer in which the cells form bridges across the main air space system (Pl. XIX, Fig. 17). All these cells contain chloroplasts in a marginal position, which have increased in size since they were first detectable as denser granules in the meristematic protoplast. The nucleus is usually slung in a central position in the cell and has one or more nucleoli.

It is extraordinarily difficult to visualize all the developmental processes which are going on in the young leaf at the same time. The development of the leaf does not proceed simply from apex to base, nor can it be said to be marginal. Cell divisions followed by differentiation go on simultaneously throughout the whole of the mesophyll. The vacuolation of the upper epidermis spreads gradually with the increase of the vascular tissue, but the vacuolation of the lower epidermis occurs considerably later and appears to be bound up with the development of the stomata. While all this is taking place, a band of cells at the margin of the leaf is differentiating into comparatively thick-walled cells from the apex downwards. This differentiation of the margin is slow to begin with, but the rate increases later so that nearly all the cells in the basal quarter of the margin differentiate simultaneously. The balance between the rate of differentiation and the capacity for extension of the marginal cells on the one hand, and the rate of differentiation of the mesophyll within is evidently an important factor in determining the size and shape of the leaf. One point that becomes clear from a consideration of these processes is that the cell-walls in the early stages of leaf development must be in a very elastic and gelatinous condition. A large amount of cell readjustment takes place throughout the phases of leaf expansion, an amount which would be impossible in the presence of anything in the nature of a rigid layer. The hardening of the cell-walls evidently takes place after the stretching of the various differentiated layers has taken place—an important point when one comes to consider the structure of the silvered plum-leaf.

The development of the vascular supply of the leaf does not show any very striking features. The xylem elements are the first to be formed in the very young leaf and later the phloem elements can be distinguished.

The young vascular bundle is early associated with a starch sheath which is developed first on the phloem side and only later completely surrounds it. Finally, the whole bundle becomes more or less embedded in thick-walled vacuolated cells.

The stages of development of a normal plum-leaf can be summarized as follows:

1. The meristematic stage, in which all the mesophyll cells are meristematic. The upper epidermal cells are vacuolating at the same time as the vascular tissue is developing. The lower epidermal cells are meristematic except for a band of cells below the vascular bundles. A gradual distinction in shape between the cells of the palisade and of the future spongy mesophyll becomes apparent. No intercellular space system is present and the development of the stomata only begins towards the end of this stage.

2. The vacuolating stage, in the early part of which cell division continues in the palisade layers but slows down in the spongy mesophyll. The stretching of the upper epidermal cells begins. The lower epidermal cells are still meristematic, and the early stages in stomatal development take place and are correlated with the first appearance of the intercellular space system of the spongy mesophyll.

3. The cessation of cell division in the mesophyll; the vacuolation and stretching of these cells throughout the leaf keeps pace with the stretching of the upper epidermal cells. The complete vacuolation of the cells occurs, the chloroplasts taking up a marginal position. The development of the intercellular space system in the spongy mesophyll and palisade is completed. The cell-walls become hardened and the stomata function for gaseous exchange.

During the early developmental stages of the leaf, the nuclear divisions follow each other rapidly throughout the whole leaf so that the nuclei pass through a very short 'resting' period before the next division begins. This 'resting' period is a growth period characterized by the synthesis of new protoplasm in both nucleus and cytoplasm together with an increase in the size of the cell itself. Lundegårdh (10) called this growth period the 'interphase' and distinguished between it and the true resting stage during which the nucleus undergoes no further divisions. In the following account of the nuclear divisions these terms will be used to distinguish the two conditions. The differences between interphase and resting nuclei may reflect to a certain extent the differences in cell metabolism which presumably take place when a cell becomes highly vacuolated after a condition characterized by dense protoplasm and small vacuoles. In the latter, the metabolism of the cell is probably mainly protoplasmic, i.e. protein, while, in the former, carbohydrates (especially in the leaf) form

the main products of metabolism. Protein metabolism appears to be correlated with frequent divisions of the nucleus. In carbohydrate metabolism, the products are rapidly removed, and this apparently involves no readjustment of the now stable relationship of cytoplasm to nucleus; the latter passes into the resting condition, after which no further division takes place.

The nuclei of the plum leaf in interphase are very large in comparison with the size of the cell, and show a dense reticulum which only differs from the surrounding cytoplasm in appearance by its extra density. This was the case with all the fixatives used, and with all the different methods of staining. In the reticulum a varying number of chromatin granules are embedded. A single nucleolus can always be distinguished, and sometimes two or three are present (Pl. XIX, Figs. 1, 3). In plum leaves, when nuclei pass from interphase to a true resting condition, a reduction in size takes place and the chromatin granules, although still present, are not so conspicuous. The nuclear divisions in normal leaves were studied in detail because a comparison of normal and silvered plum leaves showed that some of the outstanding differences could be traced ultimately to differences in the nuclear divisions.

In the earliest prophase stages, the chromatinic granules increase in number and become more conspicuous. They tend to arrange themselves in rings round the nucleoli, and fine connecting threads can usually be seen connecting them to the nucleoli and to each other. The nucleoli decrease in size and look vacuolated and form additional chromatinic granules, which can often be seen breaking off from the rest of the nucleolus. In the majority of somatic mitoses, it is recorded that the nucleolus is still present when the spireme thread is organized. For instance in *Vicia faba*, Fraser and Snell (7) note that 'the nucleolus becomes vacuolate and decreases in size, doubtless giving up material to the chromatin thread'. Then in the somatic nuclei of *Oenothera lutea* the nucleolus is visible until an even later stage, for Gates (8) says that 'during these processes (i.e. the separation-out of the chromosomes) the nucleoli remain unchanged, floating freely in the cavity until the nuclear membrane disappears, when they are suddenly dissolved and vanish'. In plum leaves, however, the nucleolus becomes indistinguishable before there is any spireme or definite chromosomes, suggesting that here, and perhaps in other very rapidly growing organs, the nucleolus represents a store of easily available chromatinic material which is wholly used up in chromosome formation. With the increase in the number and size of the chromatinic granules and the disappearance of the nucleolus, any sign of a definite arrangement of the granules is soon lost. The next stage is very characteristic of the nuclear divisions of these leaves. The granules do not become united to form an elongated spireme thread in the usual

way but become arranged in a network formation very suggestive of chain-armour (Pl. XIX, Fig. 4). After this stage, the chromosomes become separated out as definite entities, and the nuclear membrane disappears. The spindle is then formed and the chromosomes come to lie in a central position on the broadest part of the spindle (Pl. XIX, Fig. 5). There is a comparatively large number of chromosomes but the nuclei are too small to be able to make an accurate count. After the chromosome halves have split and have passed to either pole of the spindle, the latter becomes extended laterally (Pl. XIX, Fig. 7). The new cell-wall can then be distinguished as a faint granular line across the broadest part of the spindle. In the palisade cells, these new walls are almost invariably laid down at right angles to the upper surface of the leaf. The new wall gradually becomes more definite, and as it extends across the cell the spindle fibres remain attached to the extremities of the young wall as it is built up across the cell, thus giving a fan-like appearance to the ends of the new wall (Pl. XIX, Fig. 8). This method of development of the new wall is described by Bailey (1) in the cambial cells of *Pinus strobus*, and in other cells which are very much longer than broad, and in which the new wall is laid down in the longest diameter of the cell. The new walls in the palisade are not completed until some time after the daughter nuclei have become reorganized. During this reorganization, the chromosomes can be distinguished throughout almost the whole process. Nuclear divisions in vacuolating cells are of common occurrence in plum leaves. Small, but conspicuous, vacuoles are present in the dense cytoplasm of the mesophyll cells after the earliest stages. An increase in the amount of vacuolation occurs earlier, and to a more marked degree in the future spongy mesophyll cells than in the palisade cells, but nuclear divisions continue for some time after this increased vacuolation has commenced. This greater amount of vacuolation in the spongy mesophyll appears to be correlated with the falling off in the rate of cell division in this layer, and may be closely bound up with a change of metabolism from protein to carbohydrate, for it is at this stage, too, that the chloroplasts become noticeable round the developing vacuoles.

In the mature stages of the normal leaf, considerable variation is found in the size of the individual cells, and in the number of rows of palisade cells formed. One or two are usually formed, but the individual cells may be very elongated at right angles to the epidermis (i.e. four or five times as long as they are broad), or they may be only twice as long as they are broad. These differences were not bound up with the position of the leaf on the tree (i.e. sun leaf or shade leaf), for none of the leaves were under shade conditions. The same variations in the mature structure of leaves were also found by Nordhausen (11) and Schramm (12), when working on the differences between sun and shade leaves. They concluded that the

age of the tree and of the leaf (i.e. its position in the bud) were important factors in determining whether its mature structure would be that of a sun leaf or a shade leaf, and that light modified the structure only to a limited extent. In developing plum leaves, the internal conditions in each leaf appear to be important factors in determining such variations in mature structure. The formation of the palisade is bound up with a continuance of rapid cell division in which the wall is laid down in one direction only. This may take place in either one or two layers of cells in the developing leaf, but at present there is nothing to indicate which factors, whether internal or external, determine such a process. The developmental history of other types of leaves under different conditions may throw more light on this point.

During the senescent stages of the leaf, the nuclei undergo a series of transformation processes, involving a decrease in size and an accumulation of stainable material. The resting nuclei are smaller than those in the interphase stages, but show a dense reticulum in which one or two nucleoli are embedded, in addition to several smaller and less deeply stained granules. When the senescent changes set in, the nuclear reticulum is the first part of the nucleus to show any changes. It becomes much less conspicuous, and so gives a more transparent appearance to the nuclei; this makes the chromatin granules more conspicuous than they were before. These transparent nuclei are often larger than the resting nuclei, but this stage is soon followed by a reduction in the size of the nuclei which brings about a condensation of the chromatin. These granules often appear to be connected to each other by fine threads. The granules increase in size and coalesce, and the nucleus then consists of a few heavily staining granules within the nuclear membrane (Pl. XX, Figs. 19 and 20). This stage is usually characteristic of yellowing leaves. Finally, the nuclei break up into fragments and become disorganized (Pl. XX, Fig. 21) owing to the dissolution of the nuclear membrane.

While the nuclei are undergoing these senescent changes, the chloroplasts begin to change in appearance. In dark green leaves they are lens-shaped with a very definite outline when looked at under the microscope. In yellowing leaves, the chloroplasts become vacuolated and gradually lose all definite outline, and then they disappear altogether: the cells, at this stage, contain mere whisps of protoplasm surrounding a heavily stained nucleus or the structureless remains of one.

Most of these senescent changes were verifiable in fresh material, but when the cells are full of chloroplasts, it is very difficult to distinguish the nucleus with absolute certainty. However, when the chloroplasts become vacuolated it is easier to distinguish the nucleus, and these later stages in the fresh material show the same features as those just described from fixed material.

*The Development and Cytology of Silvered Victoria Plum Leaves.*

Plum-trees attacked by *Stereum purpureum* are quite characteristic in appearance. Their leaves are 'silvered', i.e. they are grey-green in colour instead of the normal dark green, and in very severe cases they may become yellowish or brown early in the season. The hyphae of *Stereum purpureum* are not present in the leaves but are to be found in the stem, usually at some distance below the branches bearing the diseased leaves. Brooks and Brenchley (6) have shown that the symptoms of silvering of the leaves can be induced by injecting healthy trees with a non-living extract of the fungus. The extract has to be injected into the tree while the leaves are expanding, otherwise no silvering effects are produced.

If the silvered leaves are examined microscopically, it will be seen that the upper epidermis has become more or less separated from the mesophyll. The space so formed is filled with air which alters the reflection of the light at the surface of the leaf, and thus gives it a peculiar silvered appearance. Various cytological peculiarities in the cells of these silvered leaves have been recorded from time to time. Smolák (13), who worked with mature leaves, found that the nuclei might be larger in diseased leaves than in normal leaves, and that they might show a variety of peculiar shapes, e.g. nuclei which were very elongated or amoeboid in form. These diseased nuclei contained fewer granules than the normal nuclei, and the granules were often absent altogether. When granules were present they were usually aggregated on the periphery of the nucleus: occasionally they were found outside the nuclear membrane, thus suggesting that chromatin migration was taking place. Smolák found that the chloroplasts were affected and showed various stages of premature breakdown. The changes he observed were held to be caused by 'a strong stimulus acting somewhere in the immediate vicinity', and not by the presence of the mycelium of *S. purpureum* in the woody tissues below. He suggested that some toxic substance, entirely unconnected with the fungus, was secreted in the leaves themselves which brought about these cytological changes. Recent work on Silver-leaf disease, especially that of Brooks and his colleagues (3, 4, 5, 6), show conclusively, however, that Silver-leaf disease of plum-trees, in this country, is normally due to invasion of the woody parts of the trees by *S. purpureum*. Güssow (9) found that the upper epidermal cells of silvered leaves were larger than those of healthy leaves, owing to the bulging outwards of the lower tangential wall when it became free from the palisade cells.

Various theories have been brought forward to explain the peculiar structure of silvered leaves caused by the action of a fungus at a distance. It has been suggested that the fungus in its growth secreted a substance which was carried up to the leaves in the transpiration stream and, either

directly or indirectly, brought about a dissolution of the middle lamella. Bintner (2) says 'the dissolution of the middle lamella is perhaps brought about by the production of some diffusible poisonous substance during the metabolic processes of the fungus and of the invaded cells of the host; this substance is then conveyed to the leaves by the water conducting channels, where it causes a change in the enzymes capable of dissolving the pectic substances of the cell-wall.' Smolák (13) held that any such action on the middle lamella was due to an enzyme secreted by the cells themselves, while Grüssow (9) suggested that the fungus secreted the enzyme which dissolved the middle lamella.

Up to the present, none of the cytological work on silvered leaves of plum has taken into consideration the developmental stages through which the leaf passes during its life on the tree. For example, it was not known at what stage in its development the silvered leaf began to diverge from the normal leaf. Also there is always the possibility that the cytological peculiarities observed by Smolák were due to senescent changes in the leaf. He compared the appearance of the nuclei in normal and silvered leaves at different seasons of the year, but without the background of knowledge of the normal nuclear development in the leaf on which to interpret his results.

The work recorded in this section of the paper is an attempt to provide the developmental and cytological history of silvered leaves throughout the season. Sections through the resting buds of trees which were badly silvered in the previous season cannot be distinguished from those of normal trees except that, in very severe cases, fewer leaves are present inside the bud. When the bud is beginning to expand and is in a very active meristematic condition, the first differences between diseased and normal leaves can be detected. The difference is centred in the nuclei of the leaves and in the nuclear divisions, which show several peculiarities. In every section, inactive nuclei of various types are present in the future palisade cells and in the spongy mesophyll cells. These nuclei are dense and heavily staining bodies which show no special organization or structure and are doubtless incapable of further division. In a large number of examples, after a nuclear division has taken place, one of the daughter nuclei becomes disorganized, apparently just after the new wall has been laid down (Pl. XIX, Fig. 14). Occasionally both daughter nuclei begin to degenerate when the new wall is only just beginning to be laid down (Pl. XIX, Fig. 13). For the most part, however, the nuclei which are not obviously degenerating show the same structure—except perhaps for a more varied range in the shape and size of the nucleolus—as in normal leaves. Frequently there is only one large nucleolus which may be slightly lobed or pear-shaped, and a few small chromatinic granules may also be present in the nuclear reticulum (Pl. XIX, Fig. 9). There is no doubt



that, at this highly meristematic stage, cell division is held up to an important extent so that the number of palisade cells per unit length is less in silvered leaves than in normal leaves. In a healthy leaf, before the expanding period has begun, the palisade cells are packed close together, and give the appearance of being pressed together and almost bulging over each other. This effect was never found in definitely silvered leaves.

Another fact which shows that the nuclear behaviour is not normal is that very often, during nuclear division, the chromosomes do not become separated out as definite entities, which always occurs in the nuclear divisions of normal leaves. In metaphase, for example, the chromosomes are often clumped together on the spindle, and appear to pass to either pole without becoming separated out as single units. Probably it is only these nuclei which undergo early disorganization, because these peculiar divisions are scattered amongst nuclei which are dividing normally, in the same way that the degenerating nuclei are scattered among the normal ones. Some of these abnormal nuclei divisions are shown in Pl. XIX, Figs. 11, 12, 13.

Passing from the highly meristematic stage, the next stage is that of vacuolation and extension. This takes place in exactly the same way as in normal leaves, but it is not until this stage is reached that the epidermal cells become gradually separated from the palisade below (cf. Pl. XIX, Figs. 9 and 10). At first this separation takes place at the corners of the palisade cells, and then, with the extension of the leaf, the whole of the upper wall of the palisade cells becomes involved until long stretches of these cells are free from the epidermis. The epidermis, however, always remains attached to the main part of the leaf above a vascular bundle. Serial sections through the leaves of a young developing bud show this gradual extension of the separation of epidermis and palisade very clearly. The leaves near the apex of the expanding bud which are still in the meristematic stage, show no separation of epidermis from palisade. The leaves below this show the stage at which large intercellular spaces only are present where the palisade cells abut on to the epidermal cells, or perhaps, a later stage in which the epidermis has become quite free above one or two palisade cells. Finally, the more expanded leaves below these show the condition in which comparatively large numbers of palisade cells have become free from the epidermis.

The separation of the epidermal cells from the palisade layer can be correlated with the holding up of cell division in the latter layer during the meristematic stage. In healthy leaves, the palisade layer keeps pace with the stretching of the epidermal cells by means of continued cell division, and by its own vacuolation and stretching, forming air spaces only in connexion with the long walls of the cells. The result of the holding up of cell division in the palisade layer of silvered leaves is

that there are insufficient palisade cells per unit length of epidermis ; consequently, the epidermis, the cells of which stretch to almost their normal extent, comes away from the palisade. The separation, therefore, is a purely mechanical process, and takes place as a result of the different relationships existing between the palisade and the epidermis compared with those existing in a normal leaf. It does not involve dissolution of the middle lamella between the cell-walls of the palisade tissue and the epidermis. In severe attacks of Silver-leaf disease, the holding up of cell division is so extensive that, in the mature leaves, large numbers of the cells of the palisade and spongy parenchyma are almost unattached to their neighbours, so that it is impossible to obtain sections of such leaves in fresh material. It has already been shown in the first section of this paper that the developmental processes of normal leaves involve a certain amount of separation of the mesophyll cells, especially in the spongy parenchyma. When the number of cells in the mesophyll is reduced as in silvered leaves, the degree of separation is naturally much more marked.

The further development of silvered leaves is essentially the same as that of normal leaves, but the consequences of the early holding up of cell division is often reflected in the mature shape of the leaves. Silvered leaves are very often deformed in various ways, e.g. (1) the mid-rib may be bent into the shape of a bow, and the lamina on either side of it, instead of being quite flat, is crinkled, and the sides of the lamina tend to face each other like the leaves of a half-opened book ; (2) the leaf may be very much less developed on one side than on the other. In some of the severest attacks, the leaves had hardly been able to emerge from the bud, and were only 2–3 cm. in length ; such leaves began to go brown soon after they had reached their mature size and the tree became leafless. That was the last season in which the tree was able to produce leaves, and the fructifications of *S. purpureum* appeared on the tree in the autumn.

Various things may happen to a silvered leaf after it has reached its matured condition : it will often remain in its silvered condition—pale grey-green in colour—for some weeks, and then it begins to show a yellowish tinge round the margin. Finally, the whole of the leaf becomes yellow and falls off prematurely. This early yellowing of silvered leaves is accompanied by an excessive accumulation of starch in the cells of the mesophyll, a fact recorded by Brooks and Storey (5). In other examples, the yellowing of silvered leaves may be followed by browning, and this may involve the whole leaf or only patches of it before it falls off the tree.

In order to give an adequate idea of the wide range of cytological behaviour found in mature silvered leaves, the observations made on some definite examples will be given. These observations can be divided into three groups:

- (1) Those on the leaves of trees in a very advanced stage of the disease

which were always very much smaller than those of normal trees. They showed yellowing and browning at a very early stage (13. v. 29 to 12. vi. 29), and fell off very prematurely, i.e. about July.

(2) Those on leaves which were deformed but more nearly approached the normal size. Yellowing and browning was present by 2. vii. 29. Leaf-fall was premature, i.e. about early September.

(3) Those on leaves which were more or less normal in shape and size, but showed heavy silvering symptoms. These leaves were still on the tree on October 8th, but they had turned yellow towards the end of September, and this was followed by some browning in small isolated patches.

*Leaves of Class 1.* The cells of the mesophyll of these leaves showed that nuclear and cytoplasmic degeneration was taking place very rapidly. The nucleus was generally reduced to a small heavily staining globule, and sometimes it was not distinguishable in the heavily staining protoplast. Some of the cells, however, were normal in appearance. Intermediate stages between normal and degenerate nuclei were of rare occurrence, suggesting that degeneration, when once it had set in, took place very rapidly. In these leaves, large numbers of palisade cells were free from the epidermal cells.

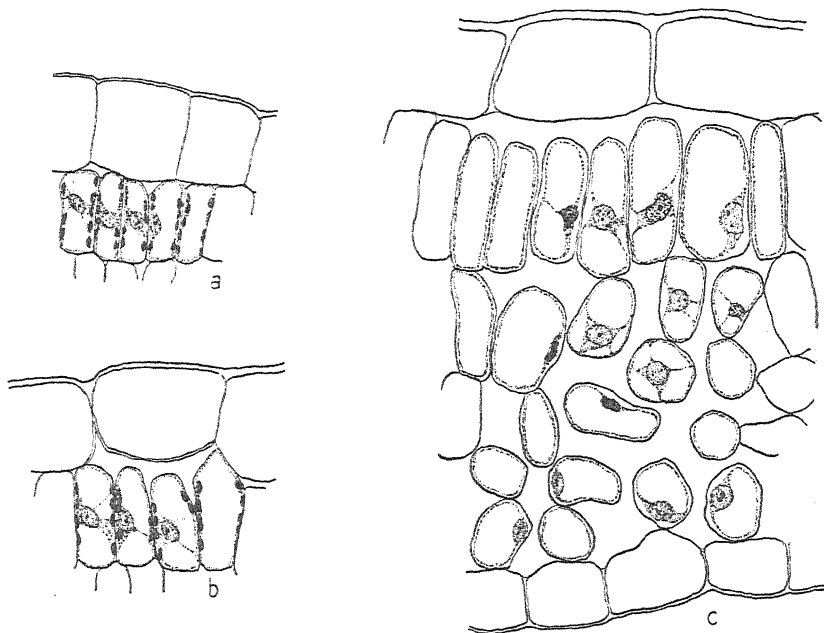
*Leaves of Class 2.* In this class, two examples will be given, in the first one of which the disease was more advanced than in the second. The cell contents of the leaves of the first example were characterized by granular deposits which completely filled a large proportion of the vacuoles of the cells. These deposits could be seen in fresh material, but they were even more prominent in preserved material owing, no doubt, to precipitation by the fixative. The nuclei of these cells—when distinguishable—were for the most part very small, and became heavily stained. Other nuclei in cells in which the deposit was less heavy, or altogether absent, were often quite normal in appearance. The chloroplasts in this leaf were small and contained no starch grains.

In the second example, no granular deposits were present in the cells, but there was an excessive accumulation of starch (Pl. XIX, Fig. 16). The majority of the nuclei were small, and became heavily stained (Text-fig. 3c); occasionally they were flattened against the long wall of the cell or were stretched across it, but some of the cells contained normal nuclei.

Leaves such as the above fell off prematurely, and were in various stages of yellowing and browning when this occurred. The microtomed preparations indicated that nuclear breakdown occurred before degeneration of the rest of the cell contents took place.

*Leaves of Class 3.* Leaves of this class were still attached to the tree on October 10th, 1929. A typical leaf was grey-green in colour with a tinge of yellow, and it had one patch of brown in the middle of the margin. Much of the palisade was free from the epidermis. The condition of the

cell contents in any one section was extraordinarily varied, and this variety was characteristic of all the silvered leaves which had remained on the tree until the autumn. Cells in which the nuclei became densely stained and



TEXT-FIG. 3. *a* and *b* Transverse sections of part of a silvered Victoria plum leaf showing stages in the separation of epidermis and palisade. *c*. Mature leaf which is heavily silvered and yellow in colour. Transverse section showing extensive separation of the cells and several degenerating nuclei. No chloroplasts are shown, but they all contained large starch granules.

the chloroplasts had disappeared were present with cells containing chloroplasts with starch grains and normal nuclei. Mixed up with these were other cells with chloroplasts devoid of starch grains and small, heavily staining nuclei. Finally, some nuclei might show the normal network structure in one half while the other half was densely stained (Pl. XIX, Fig. 15).

The leaves mentioned in the above groups were all obviously heavily silvered, although the disease was more advanced in some than in others. Leaves which are only moderately or slightly silvered rarely show any peculiar cytological features in their mature stages. They can only be distinguished with certainty from normal leaves by the separation (to a much less degree than in heavily silvered leaves) of the palisade tissue from the epidermis, and sometimes by a tendency to an undue accumulation of starch in the mesophyll. They undergo the normal senescent changes, but the leaves in which excessive starch is present turn yellow early in the season.

## DISCUSSION.

The development and cytological peculiarities of silvered leaves are fundamentally of two classes: (1) those characteristic of the meristematic stages, and (2) those characteristic of the mature tissue.

In the first class the normal rate of cell division of the mesophyll is retarded, and it is suggested that some substance is present in diseased leaves which partially inhibits nuclear division. The injection experiments of Brooks and Brenchley (6), in which the injection of a non-living extract of *Stereum purpureum* into a healthy tree in the spring produced the symptoms of silver-leaf disease, also supports this suggestion.

In the second class the nuclei show a range of behaviour which indicates that the senescent phases in diseased leaves are passed through rapidly and prematurely, although the sequence of the stages is the same in both normal and diseased leaves except in very severe attacks of the disease. For instance, large, rather transparent looking nuclei, possessing a few deeply staining granules, similar to those found in normal leaves showing early stages of senescence, are the typical form of nuclei from about early July onwards in many silvered leaves. In normal leaves this transparent stage generally does not occur until middle or late September. This stage may be maintained for some time in leaves of Class 3 before the aggregation of granules and the disappearance of the nuclear reticulum begins to take place. When this latter phase has once set in, the nuclei of heavily silvered leaves degenerate, and this is followed by a complete breakdown of the cell contents. In the leaves most severely affected, this transparent stage is not passed through at all, and the nuclei, after a very short resting stage, pass over rapidly into the heavily staining, granular condition.

Nuclei of peculiar shape as described by Smolák cannot be said to be typical of any of the silvered leaves examined. Lobed and elongated nuclei could certainly be found, but that was also true of normal leaves; their presence in diseased leaves never suggested that they were a distinguishing feature of silvered leaves. The early breakdown of the nuclei of silvered leaves shows that probably the nuclear membrane is not so stable in silvered leaves as in normal leaves. If this is so, silvered leaves with nuclei passing through a comparatively long transparent phase may perhaps show a larger number of abnormally shaped nuclei than normal leaves. It is possible, too, that changes in shape may be brought about by changes in the cytoplasm around the nucleus, and may not necessarily be due to any alteration in the nucleus itself.

The observations of Smolák (13) that the chloroplasts of silvered leaves undergo corrosion before disintegration, and that the spongy mesophyll cells of silvered leaves are longer than those of normal leaves, were

not confirmed. No marked differences could be detected in the degeneration stages of the chloroplasts in silvered and in normal leaves, except in the rapidity in which these processes were passed through in heavily silvered leaves as compared with normal leaves. The length and shape of the spongy mesophyll cells varied to such an extent in both normal and silvered leaves that it was impossible to compare them adequately.

Güssow (9) stated that the epidermal cells in silvered leaves were larger than those of normal leaves owing to the extra elongation and the rounding off of the lower tangential wall. He held that it was owing to this extra elongation that the connexion between the palisade and the epidermis is severed. This separation has been shown to be due to an inhibition of cell division in the palisade of silvered leaves in the meristematic stage. After the separation has occurred, however, the free wall-surface of the epidermal cells is likely to stretch more than one in contact with other cells. Consequently, the lower tangential walls of the epidermal cells of silvered leaves are often longer than those of normal plum leaves.

In conclusion, I should like to thank Mr. F. T. Brooks for suggesting this problem to me. I am especially grateful to him for his helpful advice and criticism while the work has been in progress.

#### SUMMARY.

1. An account is given of the development of normal plum leaves from the earliest stages until maturity. The earliest stage is characterized by a high rate of cell division. It is followed by an extension period in which a large amount of readjustment takes place among the mesophyll cells. This gives rise to the intercellular space system, and finally the mature structure of the leaf.

2. The nuclear divisions of normal plum leaves and the senescent changes in the mesophyll cells are described.

3. The development of silvered plum leaves is compared with that of normal leaves. Differences in the nuclear divisions of silvered leaves in the meristematic stages are described. The frequent inhibition of cell division in the palisade before the cessation of the extension of the epidermal cells is correlated with the subsequent separation of the epidermis from the palisade.

4. Details of the cytological features of developing and mature silvered leaves are given. It is shown that silvered leaves pass through senescent changes similar to those of normal leaves, but earlier in the season and at a much greater rate than in the latter.

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EXPLANATION OF PLATES XIX AND XX.

Illustrating Miss Tetley's paper on The Development and Cytology of the Leaves of Healthy and 'Silvered' *Victoria Plum-trees*.

The drawings were made under a camera lucida using a Zeiss 3 mm. lens, n.a. 1.25 ( $\times 1500$ ).

PLATE XIX.

Fig. 1. Transverse section of healthy *Victoria plum* leaf—stage 1. Highly meristematic stage. *u.e.*, upper epidermis; *l.e.*, lower epidermis.

Fig. 2. Transverse section of healthy leaf—stage 3. Mesophyll differentiated, and the inter-cellular space system developing.

Fig. 3. Normal nucleus of young palisade cell in interphase.

Fig. 4. Normal nucleus of young palisade cell in chain-armour stage, i.e. prophase.

Fig. 5. Normal nucleus of young palisade cell in metaphase.

Fig. 6. Normal nucleus of young palisade cell in telophase.

Fig. 7. Normal nucleus of young palisade cell in telophase, showing the broadening of the spindle.

Fig. 8. Normal nucleus of young palisade cell in late telophase, showing the new cell-wall with the remains of the spindle fibres at the extremities.

Fig. 9. Transverse section of a heavily silvered *Victoria plum* leaf in the meristematic stage showing no separation of the palisade and epidermis. Some of the nuclei have irregularly shaped nucleoli. One nucleus is in prophase (*p.n.*), and one is degenerating (*d.n.*).

Fig. 10. Transverse section of heavily silvered leaf showing the separation of the palisade and the epidermal cells.

Fig. 11. Metaphase in cell of spongy mesophyll of silvered leaf showing typical clumping of chromosomes on the spindle.

Fig. 12. Telophase in palisade cell of silvered leaf.

Fig. 13. Late telophase in cell of spongy mesophyll. The daughter nuclei are beginning to degenerate, and the new cell-wall is only partially formed.

Fig. 14. Degeneration of one nucleus (*d.n.*) after nuclear division in a palisade cell.

Fig. 15. Nuclei from palisade cells of heavily silvered senescent leaf, showing partial degeneration.

Fig. 16. Palisade cell of yellow, heavily silvered leaf showing excessive starch accumulation.

#### PLATE XX.

Fig. 17. Transverse section of a mature healthy leaf.

Fig. 18. Senescent nucleus of palisade cell at commencement of the transparent stage.

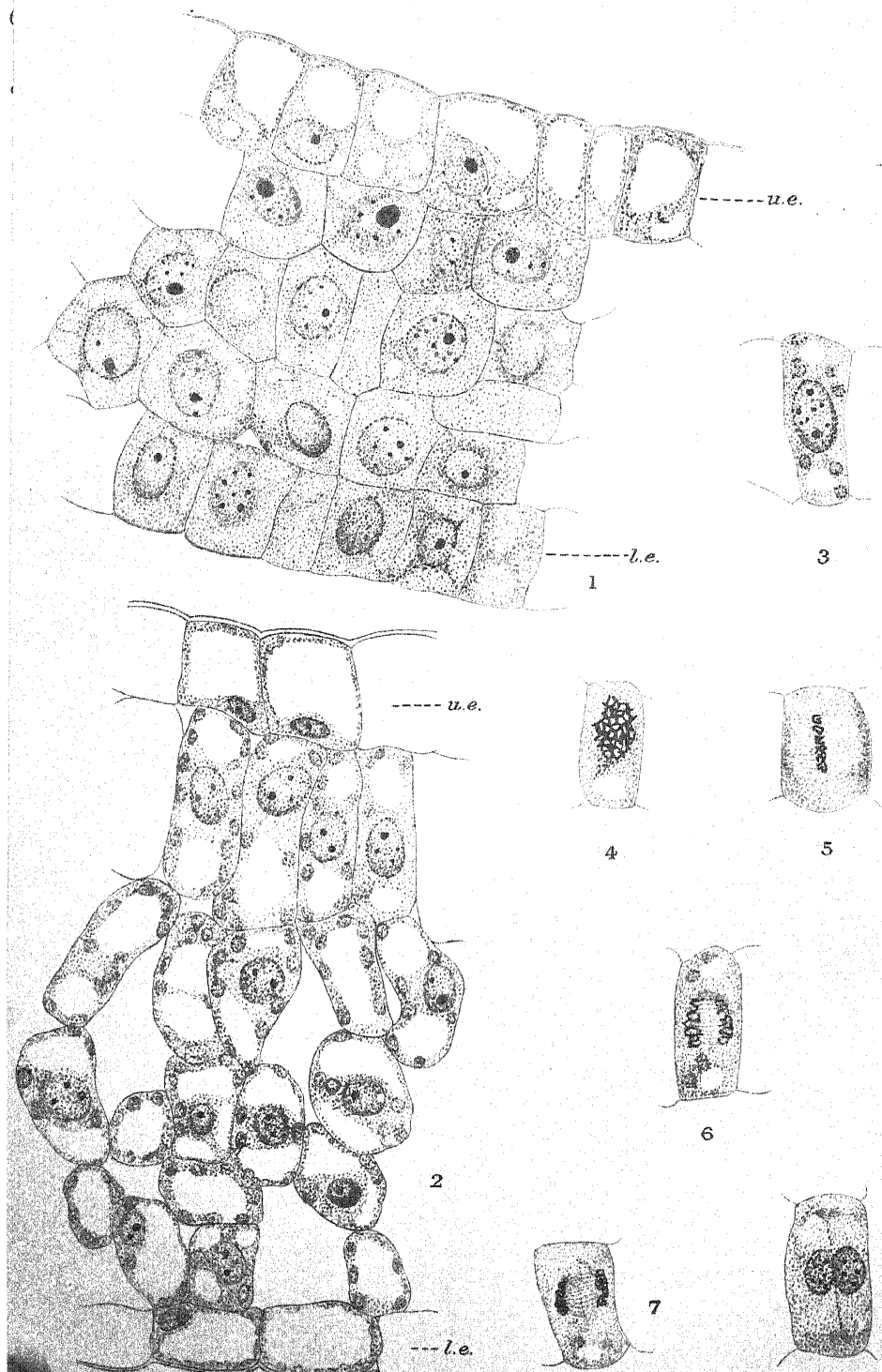
Fig. 19. Senescent nucleus of palisade cell showing the transparent and granular stage.

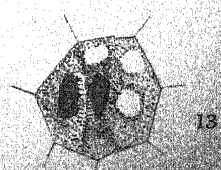
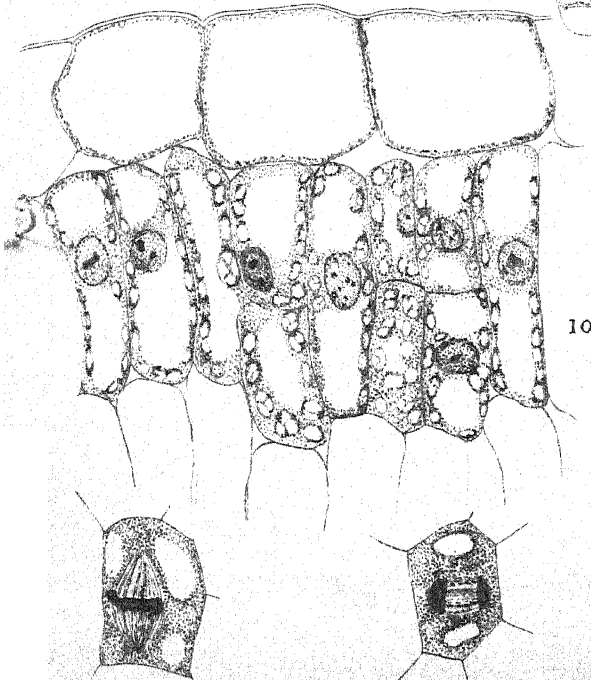
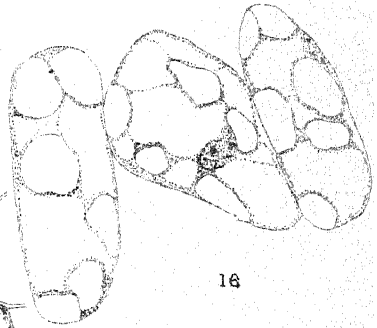
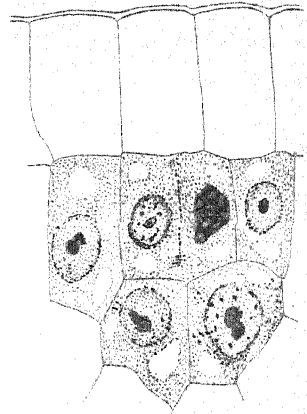
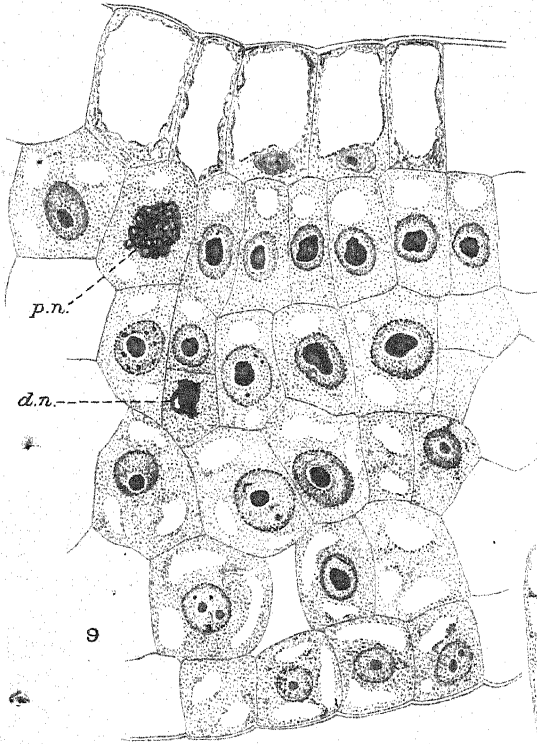
Fig. 20. Senescent nucleus of palisade cell showing condensation of granules.

Fig. 21. Senescent nucleus of palisade cell showing heavily staining stage after the breakdown of the nuclear membrane.

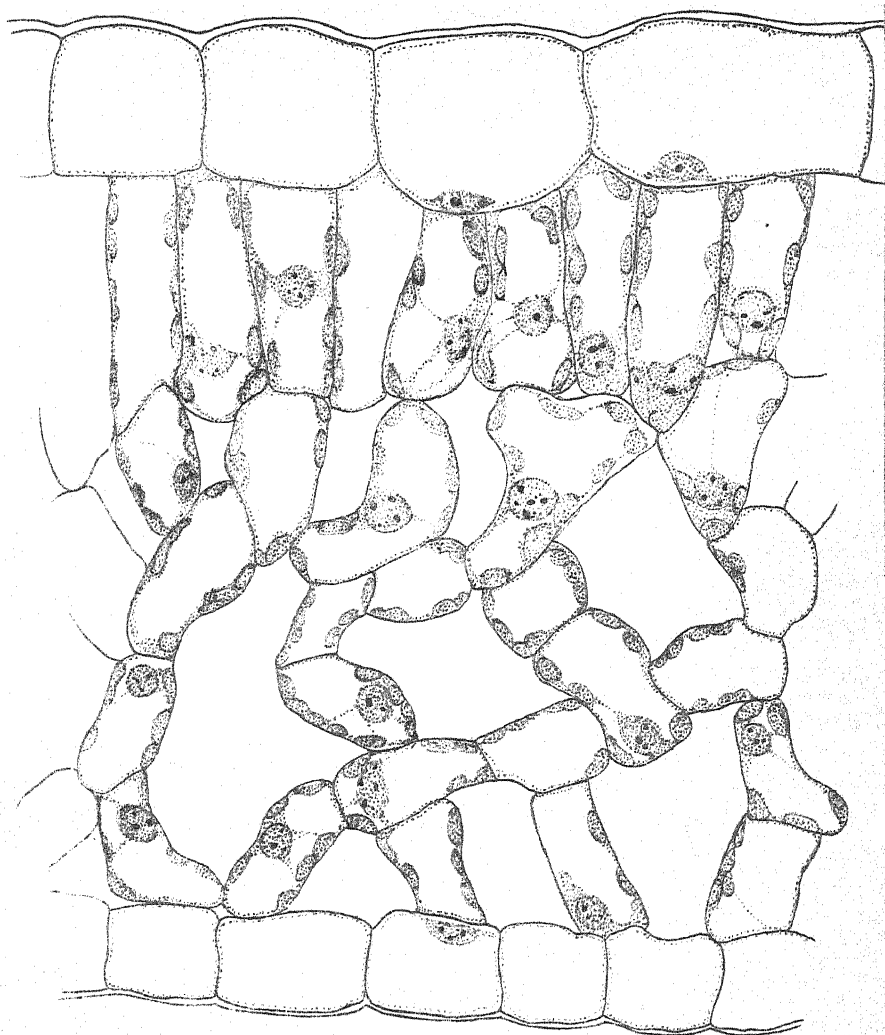




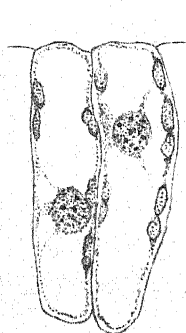




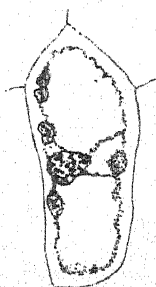
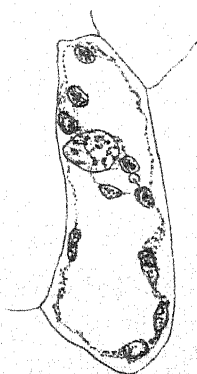




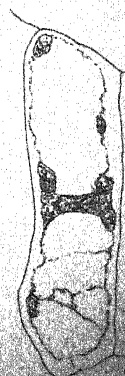
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# The Cytology and Development of *Ascobolus magnificus*.

BY

H. C. I. GWYNNE-VAUGHAN

AND

H. S. WILLIAMSON.

With Plates **XXI** to **XXIII** and thirteen Figures in the Text.

IN 1920 Dodge (4) published an account of the growth in culture of a new ascomycetous fungus *Ascobolus magnificus*. He showed that numerous accessory organs of multiplication, the papulospores, were formed, and that, in due course, provided appropriate mycelia were present, antheridia and oogonia appeared. These were brought into relation by means of a septate trichogyne, and later ascogenous hyphae grew out from the oogonium. There was evidence that the male and female organs always arose on different branches.

At the International Botanical Congress in 1930 Dr. Dodge, to whom we had spoken of the value of this work, and of our hopes that he would carry it further, told us that he had no time to continue it, and most generously offered to send us some spores. We owe him cordial thanks for having thus placed in our hands an investigation of exceptional interest.

## GROWTH IN CULTURE.

The spores dispatched by Dr. Dodge arrived on 12 November, 1930. They germinated readily, some at laboratory temperatures of 16° to 18° C., many after incubation at 25° C., and gave rise to ascocarps from which ample supplies of spores were obtained.

There is here no period of maturation after the spores are shed, such as is found in *Lachnea cretea* (8) and in *Humaria granulata* (9); they germinate at once, some while still almost colourless, others after acquiring the characteristic reddish brown colour.

The fungus was grown in Petri dishes, and flourished best on agar made up with extract of horse-dung to which, before it had set, aqueous

solution of sodium carbonate had been added, sufficient to provide a centinormal solution.

Good growth was also obtained when filter paper was added to dung agar, but in this case the proportion of papulospores was greatly increased. On agar with extract of pig-dung there was growth, but no fruits; this was also the case on Barnes's medium (7), on the medium known as M (7, 8) both with and without filter paper, and on horse-dung agar to which 0.2 per cent. peptone and 0.2 per cent. asparagin had been added. On Brown's medium (1) without glucose (asparagin 0.2 per cent.,  $K_3PO_4$  0.125 per cent.,  $MgSO_4$  0.075 per cent., agar 2.5 per cent.) vegetative growth was good, but fruits were not numerous, and contained few asci.

As the papulospores had already been investigated by Hotson (12) and by Dodge (3, 4), we avoided those media which encouraged their growth and dealt no further with them.

Ascocarps appeared on none of the media in the dark. They were most readily obtained when dishes of horse-dung agar were placed in an incubator at 25° C. for three days after sowing, and then transferred to a shelf above a radiator, where the temperature was 28° to 30° C., and where illumination was provided by two daylight electric lamps. Under these conditions sexual organs may appear within three hours of transfer, and ascogenous hyphae in thirteen hours. The strength of the dung decoction used in making up the medium is important in determining the size reached by the mature fruits. The largest obtained were on sterilized dung, over which a film of dung agar had been poured.

#### HETEROTHALLISM.

In single-spore cultures, even under the most favourable conditions, no fruits are formed. Papulospores may be abundant, and short ovoid branches, which are the rudiments of the sexual organs, may be produced. They do not develop far enough to be distinguished as male or female, but lose their contents and soon die. In mass culture, or when mycelia from suitable spores are brought together, the growth of these branches is continued, their apices become attached, the female branch, with increasing growth, is twisted round the male, and eventually an ascocarp is produced. According to their capacity to fruit together, spores and the mycelia to which they give rise are distinguished as A and B.

A and B spores cannot be recognized by their colour, by rapidity of germination, or by the sexual rudiments produced on their mycelia; indeed, the only distinction between them lies in the capacity of each mycelium to fruit with a mycelium of the opposite kind, but not with a mycelium similar to itself. A term without theoretical implications is needed, and we have found it convenient to refer to such heterothallic mycelia as complementary (11) or of different complement.



In order to ascertain whether the same fruit gave rise to A and B spores, single ascocarps, before the first spores had ripened, were attached on small blocks of inoculum to the lids of Petri dishes, and these were inverted so that the spores shot upwards on to the agar on the floor of the dish. When a good supply of spores had been shed, the lid bearing the inoculum and single ascocarp was replaced by a clean cover. Each dish thus contained untouched spores from one ascocarp only. In fifteen cases out of eighteen, spores from a single ascocarp gave rise to mixed A and B mycelia, as shown by the production of numerous fruits. In one of the remaining dishes the mycelium showed rudiments of sexual branches little more advanced than in single spore culture, in the second and third some development of sheath took place, and single sexual branches grew up and became coiled, but soon emptied. The fertile cultures fruited in three days; eight days after they had done so, when the infertile cultures were eleven days old, fresh dishes were inoculated from each of the latter, and they were grown against A and B mycelia. The first and second fruited with A but not with B, the third with B but not with A. Apparently the ascocarps from which the three infertile cultures were derived produced only A or only B spores; it may be inferred that they had arisen without the union of A and B strains.

A number of attempts was made to induce the development of the sexual branches without allowing A and B mycelia to intermingle. Petri dishes were divided by a barrier of filter paper or parchment fixed to the floor and sides by means of paraffin wax or Chatterton's mixture. Agar was poured on either side of the barrier, and inoculated respectively with A and B. In most cases one or other of the mycelia succeeded either in penetrating through the paraffin or Chatterton's mixture, or in piercing the filter paper. In the instances when this failed to occur no greater growth of the sexual rudiments was observed than in single spore-culture. Barriers of plaster of Paris were employed with the same result.

In the hope of ensuring separation, one inoculation was now made inside and the other outside a segment of porous pot. Penetration was impossible, but, when the apparatus was set up in the damp atmosphere of a closed dish, the mycelia climbed the barrier and intermingled.

To obviate this a porous pot was arranged in a deep jar; a layer of agar was poured into the bottom of the pot and into the jar around its base; the openings were carefully plugged with cotton-wool; the agar in the porous pot and the walls of the pot itself were kept moist by sterilized water introduced through a narrow tube. The mycelium in the pot grew for some distance up the walls, but ultimately the tips died off for lack of water. In this case also the complementary mycelia, separated only by the saturated walls of the porous pot, had no recognizable effect upon each other. The walls of the porous pot were tested and were found,

under the conditions of the experiment, to be readily permeable to various organic and inorganic substances in solution.

It would appear that contact is normally necessary to induce differentiation and development of the sexual branches.

#### FIXATION AND STAINING.

Material for examination was fixed in Fleming's strong solution diluted with an equal quantity of water. The young stages are easily detached from the substratum, and great care in fixing and washing is essential. When the material was abundant fixation was carried out in the Petri dish in which the fungus was growing, the dish being filled with fixing fluid and placed, if pumping was required, in a desiccator attached to an exhaust-pump. After twenty-four hours the dish and its contents were gently sunk in a large bowl of water, and removed whenever the water had to be changed. In taking up through the alcohols, and in embedding, precautions against the detachment of young specimens had also to be observed.

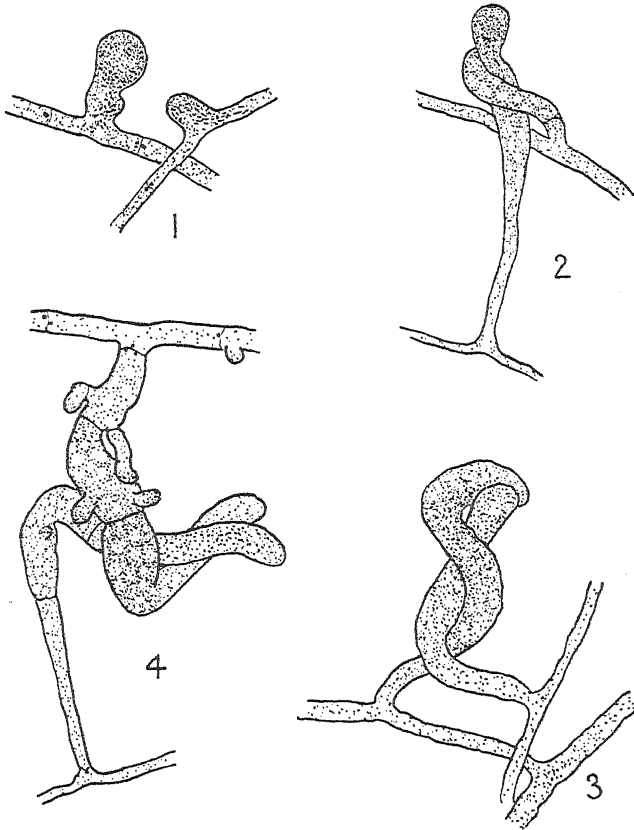
For general study material was stained in bulk in equal parts of glycerine and saturated aqueous solution of erythrosin, well washed in 50 per cent. glycerine, and carried up to 75 per cent. glycerine before mounting in glycerine jelly. By these means the sexual organs and young fruits could be observed on the substratum, a strip of agar 1 in. wide and 2 in. long being mounted on each slide, and the direction, whether towards an A or a B inoculum, being marked at either end.

For cytological investigation sections were cut  $7\mu$  to  $10\mu$  thick, and stained for the most part in Heidenhain's haematoxylin, followed by erythrosin in clove oil. In washing out, a strong solution of iron alum, 8 per cent. or more, was found to give the best results.

#### MORPHOLOGY OF THE SEXUAL ORGANS.

An examination of glycerine jelly preparations showed that the sexual organs originate, as already stated, as short, thick branches which, in the absence of the complementary mycelium, soon die. Where mycelia of different complement are intermingled most of the branches occur in pairs (Text-fig. 1), though, in the early stages, three or four are often found together; the superfluous branches are sometimes seen empty of contents at a later stage. The members of a pair elongate (Text-fig. 2), and become attached at or near their apices (Text-fig. 3). One, which can now be recognized as the female branch, elongates more than the other and, being held by the tip, is twisted about its neighbour (Text-figs. 5, 6). Soon both branches are seen to be septate (Text-figs. 4-8); the female, especially, develops numerous walls, and is differentiated into a long, terminal, septate trichogyne, a large, unicellular oogonium, and a multicellular stalk. The

oogonium increases in size and, as already shown by Dodge (4), buds out ascogenous hyphae. These, in some cases, spread upwards into the air (Text-figs. 9, 10), while in others the sheath, which has meantime been growing up, has closed over the top of the oogonium (Text-fig. 12) before the ascogenous filaments appear. The sheath is derived from the stalks

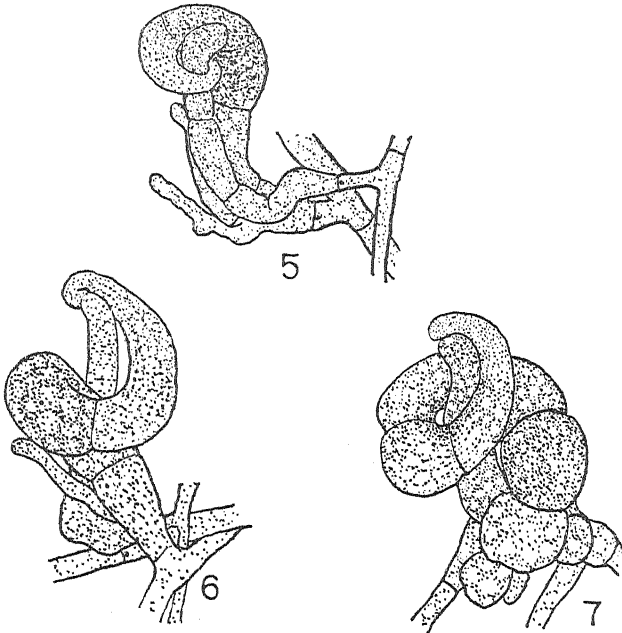


TEXT-FIGS. 1-4. Pairs of young male and female branches. In Fig. 4 the first hyphae of the sheath are growing out.  $\times 440$ .

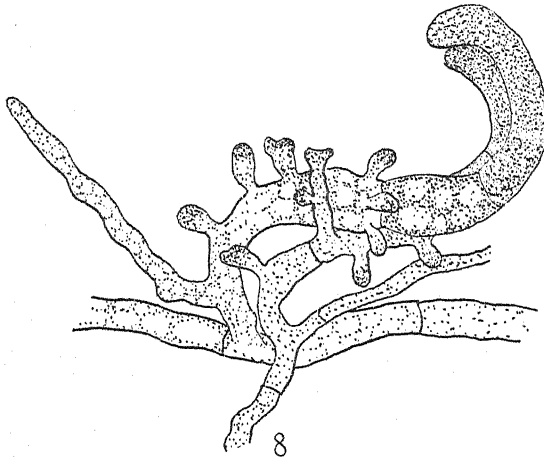
of both sexual branches (Text-figs. 4, 8), as well as, to some extent, from neighbouring cells. Text-fig. 8 shows that a balancing hypha is sometimes thrown out.

As pointed out by Dodge (4), the male and female branches are derived from different hyphae. Tracing these back, we found them to originate in mycelia from different spores, the one from an A spore, the other from a B. The obvious inference was that A and B were respectively male and female, but it was soon found that both male and female branches

could be traced both to A and to B, though of any sexual pair one was derived from each.



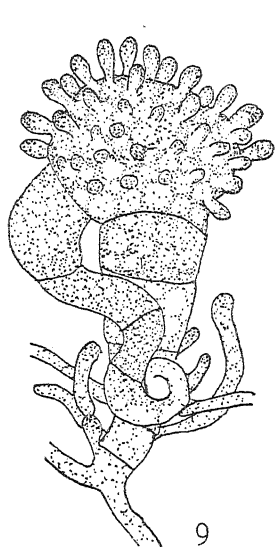
TEXT-FIGS. 5-7. Older male and female branches.  $\times 440$ .



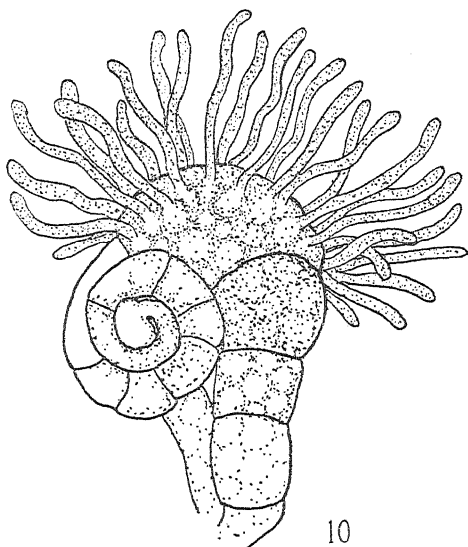
TEXT-FIG. 8. Male branch and female branch. The terminal cell of the narrower branch is the antheridium; the oogonium is the penultimate cell of the wider branch. Sheath hyphae are budding out from the stalks of both branches.  $\times 440$ .

Each of the complementary mycelia is thus found to be monoecious, bearing both male and female organs, but the male organs of one mycelium can fuse only with the female organs of the other.

In *H. granulata* (9), another heterothallic member of the Pezizales, in which oogonia, but not antheridia, are produced, and development takes



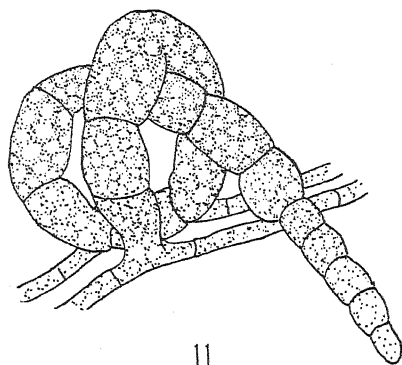
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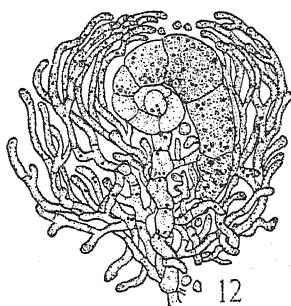
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TEXT-FIG. 9. Oogonium giving rise to ascogenous hyphae. The tip of the empty antheridium can be seen attached to the end of the trichogyne.  $\times 440$ .

TEXT-FIG. 10. Oogonium with ascogenous hyphae and empty antheridium.  $\times 440$ .



11



12

TEXT-FIG. 11. Abnormal sexual branch.  $\times 440$ .

TEXT-FIG. 12. Longitudinal section through male and female branches covered by the sheath. The figure is drawn from more than one section. The antheridium is nearly empty; in the oogonium mitosis is taking place. This figure should be compared with Text-fig. 13.  $\times 200$ .

place apogamously, mycelial fusions are common, and bring about the union of complementary mycelia. In *A. magnificus* mycelial fusions are conspicuously lacking, A and B mycelia becoming associated only through the union of the sexual organs.

In a dish infected on opposite sides with A and B inocula the sexual branches on A are female in the vicinity of the A inoculum and male at the other side of the dish, where the B inoculum is approached. Moreover, the hyphae bearing antheridia are commonly narrower (Text-figs. 3, 5, 6) than those bearing female organs. It would appear that the sexual elements tend to develop into antheridia on the distal branches of the mycelium, and into oogonia on the branches nearer the base. Areas, possibly those where the branches are much of an age, were also found in which abnormal development takes place, the antheridium being absent (Text-fig. 11) or the trichogyne failing to make contact with it. This condition has already been noted by Dodge. The fruits described on p. 655, containing only A or only B spores, may have arisen in such a region, and possibly also some of the fruits with scanty asci mentioned on p. 663.

#### CYTOLOGICAL OBSERVATIONS.

The cells of the mycelium are long, with scanty cytoplasm, except at the tips, and several nuclei each. A faintly stainable substance may be distributed throughout the nuclear area, making the nuclei difficult to identify, or material which readily takes up chromatin stains may be concentrated in two or three masses.

The young sexual branches are also multinucleate and divide into multinucleate cells, their nuclei becoming larger and clearer than those of the vegetative hyphae. Even in the very young antheridium (Pl. XXI, Fig. 1) a peculiarity of the nuclei may be recognized. Each possesses an elongated chromatin body, usually in contact with the membrane, and also a smaller, stainable granule. In the oogonium, both at this stage and later, healthy nuclei show a single, central or lateral, rounded chromatin mass.

The antheridium is the terminal cell of the male branch. It is usually oval or almost cylindrical in shape and contains, when fully grown, from 100 to 450 nuclei, each characterized by the granule and elongated chromatin body mentioned above. In a few cases a forked antheridium has been observed, recalling that sometimes present in *Pyronema confluens* (10). The stalk of the antheridium is made up of two to four cells. They give rise to branches (Pl. XXI, Figs. 2, 3) which take part in the formation of the sheath.

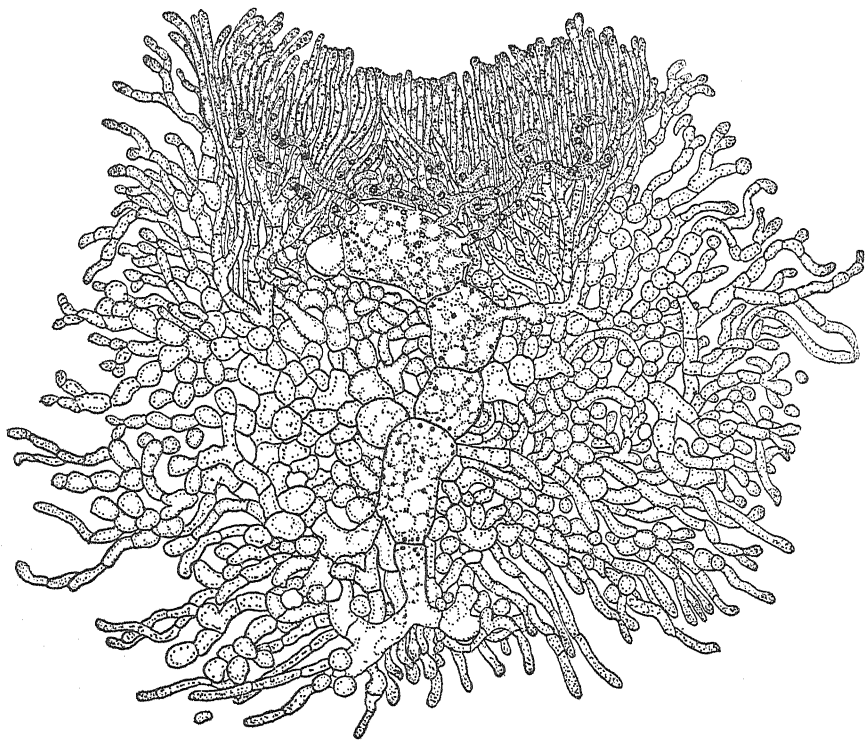
The oogonial stalk consists of three or four, or occasionally of only two cells. The lower ones resemble those of the antheridial stalk, those nearer the oogonium are larger, the uppermost stalk-cell being sometimes as long as the oogonium itself, and containing larger and clearer nuclei than the proximal cells. Branches from the oogonial stalk give rise to a considerable part of the sheath. In the young oogonium the nuclei divide

at least once during development (Pl. XXI, Figs. 5, 6), and show four chromosomes, the haploid number. After division they are arranged close to the oogonial wall, leaving a clear, central space of reticulate or finely granular cytoplasm. The number of nuclei when the oogonium is ready for fertilization varies from just under 100 to over 400, with an occasional larger specimen. In this case, as in the case of the antheridium, there is very great variation both in the size of the organ and in the size and number of the nuclei, so that it has proved impossible here to place any reliance on changes during development of the nuclear number. The trichogyne is divided into seven or more cells; they decrease in size towards the tip; their contents resemble those of the cells of the stalk.

When the sexual organs are fully grown continuity is established between the contents of the antheridium and of the end cell of the trichogyne (Pl. XXI, Fig. 2), and the nuclei begin to leave the antheridium. Owing to the presence of the granule and elongated chromatin body in the male nuclei they are readily identified at all stages of their journey. They move from cell to cell through the trichogyne, lying in each cell against the proximal wall till an aperture is formed and they travel further. The opening in the wall appears to be due to the dissolution of the central area, the region last deposited in annular wall formation, for the edges, as seen in section (Pls. XXI, Fig. 4; XXII, Fig. 8) are rounded and not ragged. Since the wall does not completely disappear, the aperture can be identified only when the knife has passed through the trichogyne or when the wall is viewed obliquely. When the trichogyne is seen from without, the wall appears intact, as in Pl. XXI, Fig. 2, and the existence of an opening can only be inferred by the presence of male nuclei in the proximal cell. As a result of the incomplete solution of the wall, male nuclei are often collected against the remaining edges as in a backwater. After the passage of the male nuclei the apical cells of the trichogyne become somewhat crushed, and are soon hard to distinguish in detail, but the larger, proximal cells retain their form, and, in their case, the breach in the wall is closed after the male nuclei have passed. Later the nuclei of these cells become swollen and their contents finally disintegrate. It is during the passage of the male nuclei through the trichogyne that the nuclei of the oogonium divide (Pl. XXI, Fig. 5); often the male nuclei have reached the cell next to the oogonium before mitosis is complete. The male nuclei crowd against the final wall (Pl. XXI, Fig. 7), it is penetrated (Pl. XXII, Fig. 8), and they pass into the female organ.

The female nuclei, their mitosis accomplished, are now lying in the periphery of the oogonium. The male nuclei reach them, and union of nuclei in pairs takes place. Owing to the presence in the female nucleus of a single chromatin body, and in the male of an elongated body and a granule, the fusion stages show three chromatin masses (Pl. XXII, Fig. 9),

two of which, towards the end of the process, are found to be in contact. Apparently it is the elongated body which unites with the chromatin mass of the female nucleus, for the fusion nuclei exhibit a smaller, rounded



TEXT-FIG. 13. Longitudinal section through ascocarp with female branch, ascogenous hyphae, and paraphyses.  $\times 200$ .

granule in addition to the ordinary chromatin body. This can be seen both in the oogonium (Pl. XXII, Fig. 10) and in the ascogenous hyphae (Pls. XXII, Fig. 11; XXIII, Fig. 15). The characteristic structure of the male nuclei makes it possible here to state definitely that in fertilization a male and a female nucleus unite. In most coenocytic fusions this can only be inferred. The nuclei have been counted in oogonia after fertilization, at stages similar to that shown in Pl. XXII, Fig. 10, and the numbers have proved to be of the same order as in the antheridia or in the oogonia before the entrance of the male nuclei. Some of the female nuclei, recognizable by their single chromatin body, are left unpaired and take no further part in development. Similarly a good many male nuclei may remain in the antheridium (Text-fig. 13, Pl. XXI, Fig. 4) or in the cells of the trichogyne and there disintegrate. The amount of this wastage varies and may no doubt be associated with the variation observed in the supply of asci in ripe ascocarps. Sometimes they are closely crowded, sometimes more



sparsely distributed, and occasionally well developed ascocarps are found which contain only a few scattered asci.

After the union of the sexual nuclei the ascogenous hyphae begin to grow out. There is much variation in the stage of development reached at this time by the sheath. The filaments which compose it may still be below the level of the oogonium, so that the ascogenous hyphae grow freely upwards (Text-figs. 9, 10, Pl. XXII, Figs. 10, 14), or the sheath may extend above the oogonium (Text-fig. 12, Pl. XXII, Fig. 11) so that they insinuate themselves among the vegetative filaments from the first. In all cases the sheath sooner or later completely encloses the oogonium (Text-fig. 13), and spreads upwards, forming a penthouse of narrow, straight branches the first of the paraphyses. They grow energetically and frequent mitoses are found in their cells. There is some evidence that the paraphyses are derived from branches of the upper cells of the oogonial stalk.

When the growth of the sheath is slight the ascogenous hyphae are narrow (Text-fig. 10, Pl. XXII, Fig. 14) and their nuclei lie in single file. This is the commonest arrangement and is reached sooner or later in all cases, but the first outgrowths of enclosed oogonia are sometimes wide, with dichotomous branching and crowded nuclei.

As in *H. granulata* (9) and in *P. confluens* (10), simultaneous mitosis takes place, before the hyphae divide, in all diploid nuclei, whether in the ascogenous hyphae or still in the oogonium (Pl. XXII, Figs. 12, 13). Often several resting nuclei are present and may be recognized by their single chromatin body as unpaired nuclei of the oogonium.

At this stage eight chromosomes can be counted passing to each pole (Pl. XXII, Fig. 13) in contrast to the four seen in divisions in the oogonium before fertilization (Pl. XXI, Fig. 5). The nuclei in the ascogenous hyphae are therefore diploid.

Septation of the ascogenous hyphae takes place as described for *P. confluens* (10), walls being formed across the relatively empty area left by the spindle between two sister nuclei (Pl. XXII, Fig. 14). As a result the terminal cell of the ascogenous hypha is uninucleate, the basal cell contains one or three nuclei, and the intervening cells contain two each in mitosis (Text-fig. 13; Pls. XXII, Fig. 14, XXIII, Fig. 15). As in *P. confluens* these nuclei are diploid; the number of chromosomes, and the structure in interphase, showing the granule from the male nucleus, alike preclude the possibility of interpreting them as unfused sexual nuclei. The binucleate cells of the ascogenous hyphae grow out laterally, the two nuclei pass into the branch and there divide, and a uninucleate end cell is again cut off. The binucleate cell thus produced may at once form an ascus or may branch again. Ascus formation is preceded by fusion of the two nuclei and follows the usual method for members of the group.

In the prophase of the first division in the ascus two deeply staining

bodies (Pl. XXIII, Fig. 16) are constantly seen. It is possible that they represent the granules, one of which was present in each male nucleus and later, after fertilization, in each nucleus of the ascogenous hyphae. They can no longer be distinguished when the gemini pass on to the spindle, for deeply staining granules are often in contact with the nuclear membrane at this stage. Later a rounded, stainable body, in addition to the usual chromatin mass, is present in the nucleus of the spore, but there are no means of identifying it with the bodies found in the male nuclei or in the meiotic prophase, and, owing to the granular character of the vegetative nuclei, it cannot be traced further. In the first mitosis in the ascus eight gemini are present (Pl. XXIII, Fig. 17), and eight chromosomes travel to each pole (Pl. XXIII, Fig. 18). In the second division also (Pl. XXIII, Fig. 19) eight chromosomes are seen. In the third prophase four recurved and elongated bodies appear (Pl. XXIII, Figs. 20, 21), which are presumably composed of two chromosomes each. On the spindle they divide, four chromosomes passing to each pole (Pl. XXIII, Figs. 22, 23), so that the ascospores have haploid nuclei.

#### DISCUSSION.

During the last few months several investigators have published work on the discomycetous fungi. In March, 1931, Green (6) gave an account of the growth of various members of the *Ascobolaceae*, and especially of the occurrence of heterothallism in *Ascobolus stercorarius*, where mycelia are of two types, A and B, and apothecia develop readily when they meet. In a few cases mycelia were found to produce fruits in monospore culture. Oidia are common, and fruits are formed when oidia of different complement are brought together. In October of the same year Dowding (5), working independently, confirmed the existence of heterothallism in *A. stercorarius* and suggested the importance in the wild state of the distribution of the oidia by flies.

In April, 1931, an account was given (10) of the fusion of male and female nuclei in the oogonium of *P. confluens* and the paired nuclei in the ascogenous hyphae were shown to be due, not to the association of the sexual elements, but to simultaneous division of diploid nuclei. After the usual fusion in the young ascus the nucleus is tetraploid and two reductions take place in the formation of the spores. Six chromosomes, the haploid number, were seen not only in the third telophase in the ascus, where a similar number had already been seen by Dangeard (2), but in mitoses in the germinating spores and in the vegetative filaments, while divisions in all stages of the development of the ascogenous hyphae showed twelve chromosomes, and twelve gemini were recorded in the definitive nucleus of the ascus. Moreau and Moreau (13), in October, 1931, published

the result of their study of the ascus of *Pyronema* on material from the same source. They were able to confirm the presence of twelve gemini in the first meiotic division, but they figured twelve also in the third telophase. They suggest that the discrepancy may be due to the six chromosomes having been counted in cut nuclei or in nuclei the fixation of which had caused adhesion of chromosomes. We find it necessary, therefore, to state categorically that the elementary mistake of failing to notice cut nuclei was not made, but that in every case in which the chromosomes were counted it was possible to focus the cytoplasm above and below the chromosome group. As to adhesions, it would be strange indeed, if adhesions occurred in all haploid nuclei and in none of those in the ascogenous hyphae or in the first division in the ascus. It is to be hoped that Monsieur and Madame Moreau will continue their study of this fungus and will place on record their observation of the number of chromosomes in the germinating spores, the vegetative cells, and the ascogenous filaments. Without such corroboration a study of the ascus is inadequate.

In February, 1931, Schweizer (15) described the development of a new homothallic species, *Ascobolus strobilinus*, on sheep-dung. Each fructification has several pairs of sexual organs; the male nuclei pass through a unicellular trichogyne to the oogonium and are stated there to pair, but not to fuse with the female nuclei. Male nuclei can be recognized by their small size, and the oogonium and ascogenous hyphae are described as containing nuclei in pairs, one small male, and one large female nucleus. Mitosis is not described or the number of chromosomes recorded at any stage. The Ascomycetes are a most variable group, and it would be rash to say without examination that any particular process does not occur, but the absence of nuclear fusion in the oogonium of this fungus cannot be regarded as established till confirmed by chromosomes counts, and till active material, in which nuclear division is in progress, has been searched for the sexual fusion. The size of fungal nuclei is usually too variable to form a useful criterion alone, especially as, after normal fertilization, both large, fusion nuclei, with the diploid chromosome number, and small, haploid ones are to be found in the oogonium.

Our study of the cytology of the fungi during the last few years has brought home to us the importance of active material, as indicated by the presence of mitoses either in the sexual organs and their products or in the paraphyses and other vegetative cells. It is essential to ascertain the conditions of temperature, food, and illumination by which active growth is encouraged in the fungus under observation, and to select for fixation a period of active development. The mitoses in the young ascogenous hyphae of *A. magnificus*, for example, were obtained only after more than a year's experience of the production of ascocarps in culture. When the

required conditions were known, a series of hour to hour fixations was carried out.

Equally important is the fixation of undisturbed material and the employment of rapid methods. If material is removed from the warmth and light in which it is growing to the laboratory bench in preparation for fixation, any fusions and divisions in progress may well have been completed before the fungus is killed and others will certainly not begin. In the case of young material it is well to flood the Petri dish or other vessel in which it is growing with fixing fluid while still in the accustomed position.

*A. magnificus* provides a fresh example of a member of the Pezizales with normal fertilization. As in *P. confluens* (10), union of the male and female nuclei occurs in the oogonium, and the ascogenous hyphae contain nuclei with the diploid number of chromosomes, while the definitive nucleus of the ascus is tetraploid. It is, moreover, the first case in which fertilization has been observed in an organism with a septate trichogyne, and it makes evident that the complexity of this appendage is no bar to the passage of the male nuclei.

*A. magnificus* is, also, peculiar in the elongation of the chromatin body of the male nuclei and in the presence, in addition, of a deeply staining granule by which they can be recognized at all stages. The persistence of the granule after fertilization in the fusion nuclei confirms the occurrence of nuclear union in the oogonium and makes it possible to distinguish between diploid and haploid nuclei in interphase.

The thallus of *A. magnificus* is monoecious, all thalli being capable of bearing both male and female organs, but it is not homothallic. The sexual branches can only pass beyond the rudimentary stage, and mature fruits can only be formed when two complementary mycelia are intermingled. Such a mycelial difference has been described in other Ascomycetes, in the Basidiomycetes, and elsewhere as a distinction of sex. This interpretation is not justified in *A. magnificus*, where both mycelia bear identical male and identical female organs. The difference is comparable to that between thrum-eyed and pin-eyed primulas, where both bear stamens and carpels but neither is commonly fertile alone. In *Ascobolus*, however, there is no evidence of the occurrence of even exceptional endogamy.

In *H. granulata* (9), in *A. stercorarius* (6), and in other heterothallic forms which lack an antheridium but bear female organs on both + and — mycelia, the distinction is doubtless of the same character as in *A. magnificus*; it is one of complement and not of sex.

It seems inevitable to extend this reasoning to the numerous heterothallic species in which both male and female organs are absent, and to say that, here also, while sex has wholly disappeared, a difference of complement has been established.

Just what this difference means, and how it has come into existence

remain yet to be discovered, but it may tentatively be suggested that its occurrence in monoecious forms provides that association of hereditary material from different individuals which gives opportunities for variation. Its appearance may have been followed by the loss, or partial loss, of the sexual apparatus, but it is not unlikely that it arose in different species at diverse stages in the disappearance of normal sexual reproduction. In *A. magnificus* it evidently developed while the male and female organs were still functional, and it operates through them. In *H. granulata* it may be surmised to have become established after the antheridium had ceased to function, the association of complementary hyphae being achieved through mycelial fusions which result in the effective development of the oogonium. In the Hymenomycetes, where, also, the association of the complements is mycelial, its appearance was perhaps delayed till the oogonium as well as the antheridium had been lost. It may be surmised that, though morphologically such unions are distinct from sex, they have physiologically the same effect.

In the Mucorales, the first group in which heterothallism was described, speculation is more difficult. Since the gametangia do not differ in structure, it is impossible to say whether the case is analogous to that of *A. magnificus*, or whether, in this instance, the difference between + and - is truly one of sex. The heterogamic members of the Mucorales, in which dioecism might most readily have been expected, are in every case monoecious, and it is not impossible that monoecism is universal in the group. Differences of size occur in the gametangia which unite in heterothallic forms, and both larger and smaller gametangia occur both on + and - mycelia, so that, if the arrangement is of the type found in *A. magnificus*, the difference in size may be an indication of sex.

There is great need for the detailed study of cytology in additional heterothallic forms.

#### SUMMARY.

1. Spores of *A. magnificus* were received from Dr. B. O. Dodge, of the New York Botanical Garden, to whom sincere thanks are tendered. They germinated without a period of rest and proved to be of two kinds, A and B.

2. Both A and B mycelia produced papulospores, but were otherwise infertile in single spore culture. When intermingled they gave rise to ascocarps. The fungus is therefore heterothallic. No difference could be recognized between A and B spores or mycelia, except in their capacity to fruit with the opposite strain.

3. Both A and B mycelia bear sexual branches, but these do not develop except when both strains are present, when they become

differentiated as male and female. Male branches are borne on the younger, female branches on the older hyphae of both strains. The difference between A and B is therefore not one of sex. It has been found convenient to use the terms *complementary* and *of different complement* to indicate this distinction. A antheridia will unite only with B oogonia, and B. antheridia with A oogonia.

4. The male branch has a stalk of two to four cells and a terminal antheridium. The antheridial nuclei are distinguished by an elongated chromatin body and a stainable granule.

5. The female branch ends in a septate trichogyne of seven or more cells. There is a large, unicellular oogonium and a septate stalk. The oogonial nuclei show a single, rounded chromatin body. They undergo mitosis showing four chromosomes, the haploid number.

6. The male nuclei pass through the cells of the trichogyne, apertures being formed in the walls, enter the oogonium, and fuse in pairs with the female nuclei.

7. Ascogenous hyphae now bud out, their nuclei are diploid, showing eight chromosomes. In interphase a granule like that of the male nucleus, and presumably derived from it, is present in addition to the usual chromatin body. Walls are formed between sister nuclei, and each hypha shows a uninucleate terminal and basal cell and intermediate cells with two nuclei. The binucleate cells may bud out to form branches and finally asci. In ascus formation two nuclei fuse.

8. The sheath is derived from the stalks of both sexual branches. It may cover the oogonium before fertilization, or may be relatively slow in development, so that the ascogenous hyphae at first grow freely into the air.

9. The first division in the ascus shows eight gemini in metaphase; in the anaphases of the first and second divisions eight chromosomes pass to each pole. Four recurved and apparently double bodies appear in the third prophase, and four chromosomes, the haploid number, are distributed to each of the daughter nuclei, about which the spores are formed.

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## EXPLANATION OF PLATES XXI TO XXIII.

Illustrating Professor Dame Helen Gwynne-Vaughan and Mrs. Williamson's paper  
on the Cytology and Development of *Ascobolus magnificus*.

### PLATE XXI.

Fig. 1. Young male and female branches; the former, on the right, nuclei show the granule and elongated chromatin body.  $\times 1,000$ .<sup>1</sup>

Fig. 2. Antheridium in continuity with trichogyne, into the cells of which several male nuclei have passed.  $\times 1,000$ .

Fig. 3. Nearly empty antheridium with coils of trichogyne cut across and containing male nuclei. Branches have grown out from the antheridial stalk.  $\times 1,000$ .

Fig. 4. Male and female branches. The antheridium is nearly empty. Openings are visible in several walls between the cells of the trichogyne, which contains male nuclei; the oogonium is enlarged.  $\times 1,000$ .<sup>1</sup>

Fig. 5. Oogonium shortly before fertilization with nuclei in mitosis. Male nuclei can be seen in a narrow cell which forms part of the trichogyne.  $\times 1,000$ . A single nucleus in metaphase showing four chromosomes, and one in anaphase with four going to each pole.  $\times 2,600$ .

Fig. 6. Telophase in oogonium before fertilization.  $\times 1,000$ . A single nucleus in telophase.  $\times 2,600$ .

Fig. 7. Oogonium and proximal cells of trichogyne. The male nuclei in the last cell of the trichogyne are pressing against the oogonial wall.  $\times 1,000$ .

### PLATE XXII.

Fig. 8. Oogonium and proximal cells of trichogyne. An opening is seen in the wall between the trichogyne and oogonium, and the male nuclei are passing through; female nuclei lie around the periphery of the oogonium.  $\times 1,000$ .<sup>1</sup> A group of male nuclei.  $\times 2,600$ .

Fig. 9. Fusion of sexual nuclei in the oogonium. The male nuclei show a chromatin body and a granule, the female nuclei a chromatin body only.  $\times 1,000$ . Nuclei showing three stages of fusion.  $\times 2,600$ .

<sup>1</sup> Figs. 1, 4, and 8 are drawn from more than one section.

Fig. 10. Oogonium with ascogenous hyphae beginning to bud out. In this and subsequent figures the diploid nuclei in interphase show a granule as well as the usual chromatin body; the sheath has not yet grown up.  $\times 1,000$ .

Fig. 11. Oogonium with ascogenous hyphae insinuated between the cells of the sheath.  $\times 1,000$ .

Fig. 12. Simultaneous mitosis in diploid nuclei of oogonium and ascogenous hyphae. The nuclei in the outer hyphae are slightly in advance of the others.  $\times 1,900$ .

Fig. 13. Simultaneous mitosis in nuclei of an ascogenous hypha. The nuclei are in anaphase and show eight chromosomes passing to each pole.  $\times 2,600$ .

Fig. 14. Part of an ascogenous hypha, older than those in Fig. 13. The terminal cell contains one nucleus, the proximal cells two. In each nucleus the granule shows clearly in addition to the chromatin body.  $\times 1,900$ .

#### PLATE XXIII.

Fig. 15. Oogonium giving rise to ascogenous hyphae younger than that in Fig. 14. Septation has just begun. The binucleate cells and the terminal uninucleate cell can be seen.  $\times 1,000$ .

Fig. 16. Prophase of first division in the ascus. The spireme is double, and two somewhat elongated granules are present.  $\times 2,600$ .

Fig. 17. Metaphase of first division in the ascus showing eight gemini. One of these, on the far side of the spindle, is seen from behind as a dark line.  $\times 2,600$ .

Fig. 18. Anaphase of first division in the ascus, showing eight chromosomes on the way to each pole.  $\times 2,600$ .

Fig. 19. Anaphase of second division in the ascus, showing eight chromosomes on the way to each pole.  $\times 2,600$ .

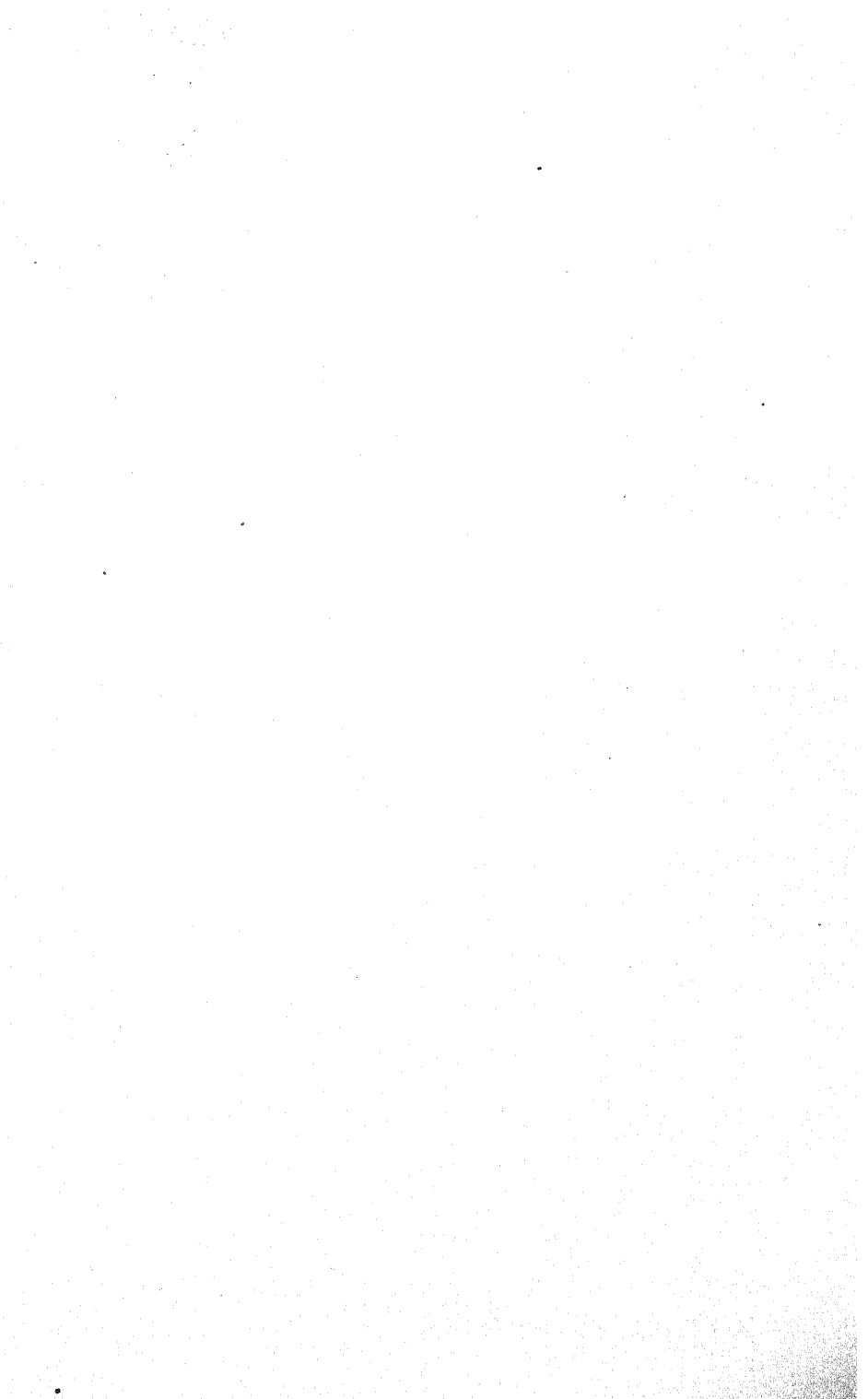
Fig. 20. Prophase of third division in the ascus. In each nucleus are four bodies which are bent over and appear to be double.  $\times 2,600$ .

Fig. 21. A later stage of the third prophase in the ascus with two nuclei each showing four double bodies.  $\times 2,600$ .

Fig. 22. Anaphase of third division in the ascus, showing four chromosomes on the way to each pole.  $\times 2,600$ .

Fig. 23. Telophase of third division in the ascus, showing four chromosomes at each pole of the spindle.  $\times 2,600$ .



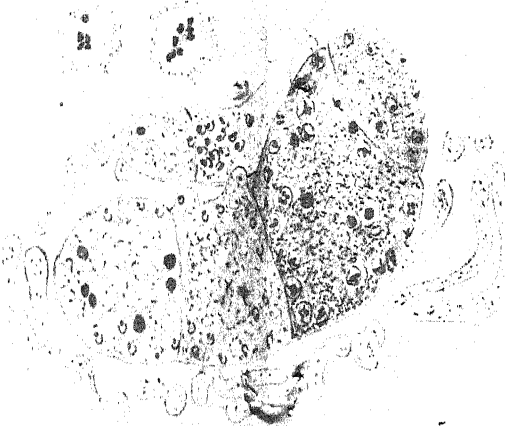




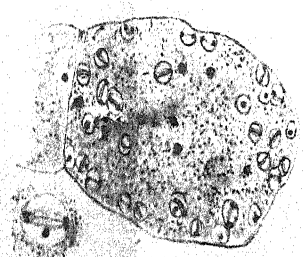
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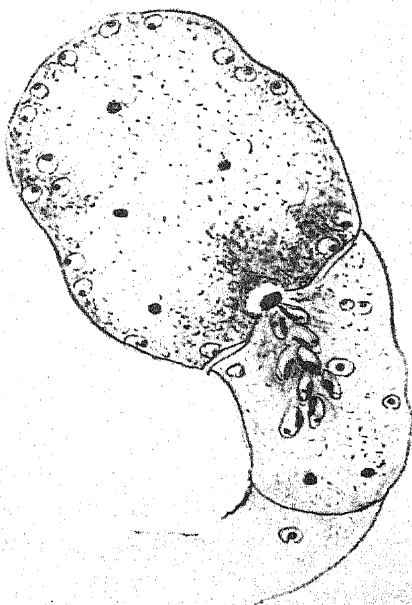


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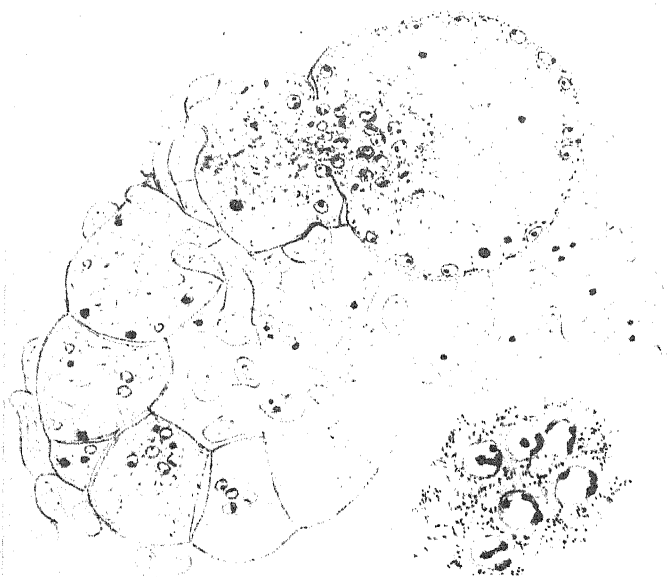




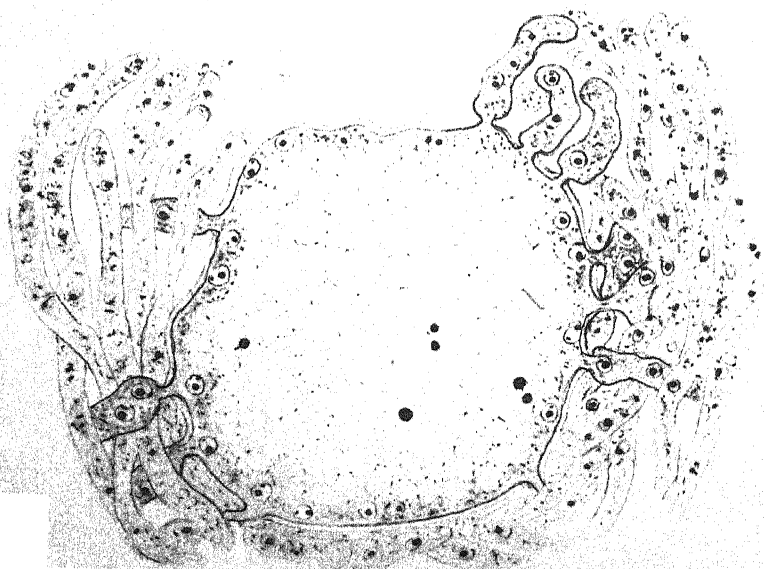
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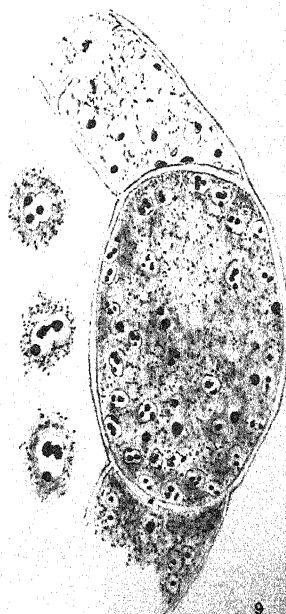
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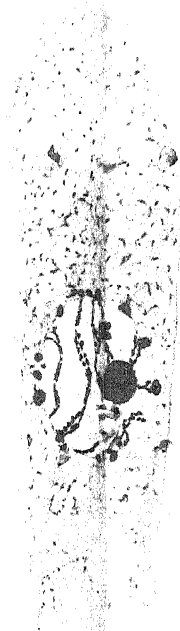
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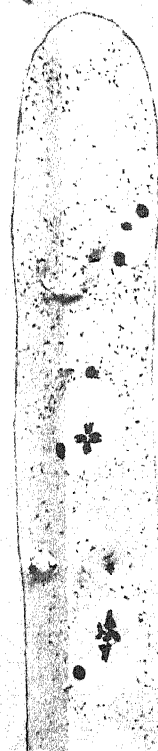
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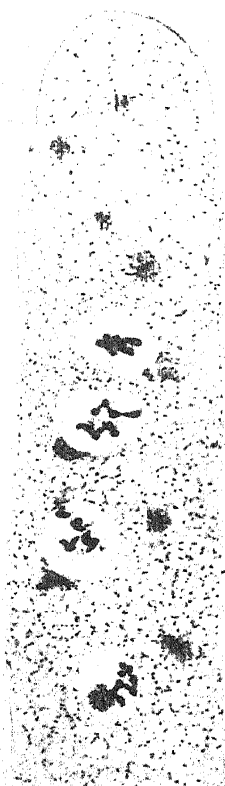




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## Studies in Growth and Differentiation.<sup>1</sup>

### II. A Preliminary Survey of the Morphology and Anatomy of *Kleinia articulata*, Haw.

BY

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AND

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With Plate XXIV and four Figures in the Text.

ONE of the impressions left by a study of the development of the stem of the Sunflower (11) has been that the parenchyma is relatively passive even from an early stage in the transverse expansion of the stem. On the other hand, it has been generally inferred from the evidence of tissue tensions that the parenchyma is active in the elongation of stems. Etiolation phenomena indicate clearly that transverse and longitudinal growth are influenced differently by light. Some observations by E. Rübel (9) suggest, too, that differences in internode length may be characteristic of different strains of Sunflowers and be inherited independently. In the succulent stem of *Kleinia articulata* parenchyma bulks more largely, and must, it would seem, take the active part in transverse as well as longitudinal growth. Moreover the contrast between the form of etiolated and normal shoots is, as usual in succulents, extreme.

In undertaking a study of *Kleinia articulata* some years ago, this problem of the relation between elongation and growth in thickness helped to determine our choice of this plant. The contrast between it and the Sunflower, both of them Compositae, offered also an opportunity of throwing light on the general problem of succulence. A closer analysis of the differences in organization and development between a herbaceous and a succulent stem is clearly desirable. Since, however, metabolic differences are involved, the characteristics of the metabolism of the plant could not be ignored.

<sup>1</sup> The first paper in this series was published in 1922, viz. D. Thoday: On the Organization of Growth and Differentiation in the Stem of the Sunflower. *Ann. Bot.*, xxxvi. 489-510.

Examination of the material available in the greenhouse in the early stages of the investigation showed a surprisingly low acidity. This result, together with the fact that the cell-sap is not at all mucilaginous, led to a special investigation of the sap solutes and the acidity changes. This has in turn provided data bearing on the problems of differentiation.

Meanwhile variations in the material have led to an extensive investigation of its behaviour under a range of cultural conditions.

In the present paper an account is given of the salient morphological and anatomical features of the plant, to serve as a general basis for the accounts in later papers of the different aspects of these investigations.

Only meagre references to the anatomy of *Kleinia articulata* have been found in the literature. Boosfeld (3) mentions a few points in a general survey of the anatomy of stem-succulents. J. Müller deals briefly with it in his dissertation on the anatomy of some woody and succulent Compositae (8): not all his observations appear to be of general validity. On the general morphology and biology of the species it will suffice here to refer to Kerner von Marilaun's account and figure (5, ii, 822-3, Fig. 455).

#### EXTERNAL MORPHOLOGY.

The shoot system (Pl. XXIV, Figs. 1 and 2) is made up of cylindrical succulent branches about a centimetre in diameter, each attached by a short constricted woody articulation, which is rather easily disjointed (5, ii, p. 823). In the resting condition the branches are leafless, often more or less uniform in diameter, blunt and rounded off at the apex, reminding one of strings of grey-green sausages of varying lengths (Pl. XXIV, Fig. 2), sometimes so short as to be spherical.<sup>1</sup> In the growing season the paler but greener shoots taper and bear numerous pale green leaves on long petioles (Pl. XXIV, Fig. 1). This appears to be the only species of *Kleinia* with petiolate leaves. Towards the end of the growing season the leaves wither, the tip rounds itself off (Pl. XXIV, Fig. 2), and at the same time the whole stem shows some further increase in diameter.

In the greenhouse the resting period is usually from about the end of April to mid-September. The young terminal inflorescences first appear about the middle of October: their axes remain short until the capitula are ready to open, and then rapidly elongate (Pl. XXIV, Fig. 5). The first capitula open late in November and flowering may continue until January. After flowering the axis droops and withers.

If an inflorescence aborts, an axillary bud near its base grows out and forms a continuation of the parent axis, only a slight constriction marking the junction between the two parts of the resulting sympodium (Pl. XXIV,

<sup>1</sup> Marloth (6, p. 237) seems to imply that plants growing on the Karroo normally have ovoid or spherical joints, but specimens at Kew hardly bear this out.

Fig. 1). A set-back in the middle of the growing season results in a very similar local reduction in diameter of a mature monopodial stem.

The leaves have mostly a broad hastate lamina, but they show a considerable range of form. The first leaf or two on a branch are often swollen and club-shaped, transitional leaves obovate or spatulate, and not lobed like the later leaves. The inflorescence axis only bears one or two linear bracts like those of the inflorescence. The under-surface of the leaves develops anthocyanin hypodermally, which becomes conspicuous in the wilting leaf. There is a waxy bloom on stem and leaves which is easily rubbed off. At each leaf-base a small brown pad is formed which remains after the leaf is shrivelled. From it three diverging lines mark the course of three leaf-trace bundles. The phyllotaxy is approximately  $2/5$ .

Rhizomes (Pl. XXIV, Fig. 3), to which no reference has been found in the literature, begin to develop about February, but become more abundant towards the end of the growing season. They are confined to the upper five centimetres of the soil, and mostly follow a horizontal course. They vary considerably in thickness. Some are thick and succulent like the aerial stems. More often they are slender, sometimes even comparable in diameter with etiolated shoots. Variations in diameter at different points along the same rhizome are common. Eventually the tip of the more slender rhizomes turns up and forms an aerial shoot. Other rhizomes become blunt-ended, probably towards the end of the growing season, and from them axillary branches develop later. The rhizomes bear small scale leaves, spirally arranged, and on the underside scattered adventitious roots. Cuttings of aerial shoots root themselves much more abundantly. Detached branches lying on the soil readily become rooted (cf. Marloth (6), p. 341).

The plant is particularly sensitive to shading. Partial shade results in elongation and smaller diameter. In deep shade very slender shoots are formed (Pl. XXIV, Fig. 6), often less than two millimetres in diameter, the leaves of which have slender translucent petioles and very small green laminae. Even in complete darkness there is little, if any, further diminution in dimensions of stem or leaves.

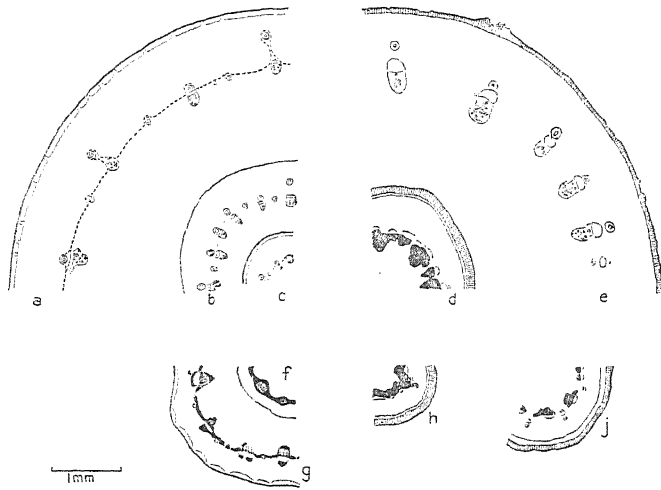
In a warm Wardian case in which the temperature did not fall below about  $15^{\circ}\text{C}$ ., plants continued vegetative growth indefinitely, in one specimen from November 1926 until December 1928. This plant had branched freely, and one branch measured over 80 centimetres in length. Miller (7, i) describes the plant as reaching three feet high or more, presumably in gardens in summer.

#### ANATOMY.

*Aerial Stem* (Text-fig. 1, *a, b*; Pl. XXIV, Figs. 7, 8). The outstanding features of the anatomy of the ordinary mature aerial stem are the large colourless pith, the green cortex, the small size of the vascular bundles,

the poverty in mechanical tissue, and the thinness of the walls of the parenchyma generally.

The surface of the stem is covered with a granular waxy bloom. The cuticle thickens with age (Text-fig. 2). There is a distinct cellulose inner



TEXT-FIG. 1. Parts of transverse sections of aerial stems and rhizomes drawn to uniform scale: *a*, mature aerial stem (broken line represents starch-sheath); *b*, young aerial stem; *c*, etiolated stem (broken line = endodermis with Casparian strip); *d*, *e*, *h*, *j*, rhizomes; *f*, top of peduncle; *g*, base of peduncle. Resin canals represented by ring; periderm shaded with radial lines, primary xylem with oblique lines, other lignified tissues cross-hatched except in *f* and *g* where lignified parenchyma is black.

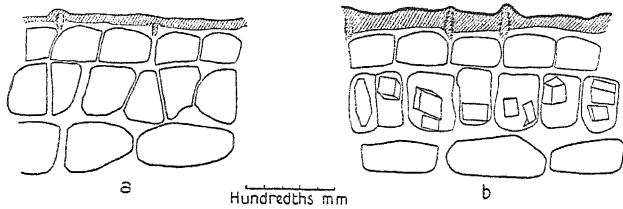
layer to the outer wall of the epidermis<sup>1</sup> under the cuticle. The epidermal cells are small and cubical, except over the leaf-traces for a short distance below the leaf-base, where they are somewhat elongated. In these same regions stomata are wanting. Elsewhere the stomata average 30 per sq. mm., are longitudinally orientated and slightly depressed below the epidermal surface. Round the margins of the depressions wax accumulates in older stems. The cuticle extends through the stomatal pores to the inner walls of the guard-cells.

Respiratory cavities interrupt a zone of collenchyma from one to three cells deep. Each cell of this zone in the mature stem contains one or two (occasionally more) conspicuous rhombohedral crystals of calcium oxalate (Text-fig. 2*b*; Pl. XXIV, Fig. 9). This is a very constant and striking feature to which we shall return subsequently. The cells are not, or only slightly, elongated, but are usually smaller radially than tangentially. They contain no chlorophyll.

Within the collenchyma is assimilating tissue of very thin-walled,

<sup>1</sup> According to von Mohl (*vide de Bary* (1), p. 77) and van Tieghem (12, i, p. 599) in *Kleinia neriifolia* the whole outer wall is cutinized, but we find a cellulose layer in this species also.

isodiametric cells. The outer and inner cells are smaller than those between, and the chloroplasts in them correspondingly more densely aggregated. All the principal bundles are accompanied on their outer side by a conspicuous



TEXT-FIG. 2. Epidermis and collenchyma of aerial stem in transverse section. *a*, young, nearly full-grown stem; *b*, old stem with thick cuticle and further-developed collenchyma with crystals of calcium oxalate. Cuticle shaded.

resin duct. There is a continuous starch-sheath which loops round the outside of the resin ducts (Text-fig. 1*a*; Pl. XXIV, Fig. 7): in fresh material the starch only stains with iodine after treatment with a swelling reagent (chlor-zinc iodine or Schimper's solution, for example, reveals it). A Casparian strip, on the other hand, has been observed locally between the resin canal and the phloem: how far this is general we cannot at present say, but the divergence between starch-sheath and layer with Casparian strip is clearly a matter for further investigation. Although no Casparian strip has been demonstrated between the bundles, the starch-sheath apparently interrupts communication between the air-space systems of cortex and pith. It is not easy to be sure how complete the interruption is; but we have not seen radial air-spaces penetrating the starch-sheath itself, although narrow longitudinal spaces occur between it and the next cortical layer, and in parts also on its inner side. The size of the air-spaces in the cortex increases rapidly outwards, and they form a well-developed system with a tendency to radial orientation. Inwards from the starch-sheath the air-spaces again increase in size. Between the isodiametric rounded cells of the pith they are uniformly distributed with the usual form of rather narrow channels, triangular in section; but they are numerous, and together hold so much air that it is impossible to assume without experimental evidence that even the innermost cells are inadequately provided with oxygen. In fact, the air-space system of *Kleinia articulata* lends no support to the view that the acid metabolism of this succulent is causally related to a deficient oxygen supply *via* incomplete respiration. Especially is this the case in older stems in which the pith becomes transversely fissured at frequent intervals.

This fissuring, together with other indications of strain, is evidence that the pith does not take an active part in the later phase of expansion in diameter that occurs in the latter part of the growing season.

The vascular bundles vary considerably in size, but they are all small

and widely spaced in the ring. The xylem includes rather a high proportion of parenchyma. There are often two or three short parallel rows of vessels, with parenchyma between them, and even the vessel rows are commonly interrupted by parenchymatous cells. The smaller bundles may only have one lignified element, or the innermost protoxylem vessel may be separated by several parenchymatous cells from the next vessel. The earliest vessels are narrow (average  $7\mu$ ) and annular, the later ones wider (average  $14\mu$ ) and spiral.

In fresh material all the vessels take up stains from very dilute solutions with remarkable avidity (congo red, safranin, bismarck brown, neutral red, &c.).

The phloem consists of narrow sieve-tubes, with short segments, and companion cells, interspersed with larger parenchymatous cells.

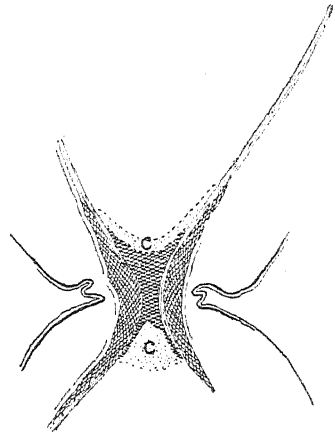
The fascicular cambium seems usually to become vacuolated in the mature stem. Between the bundles there are indications of a semi-meristematic condition of the vacuolated cells just within the starch-sheath, and in parts some cell-division (Pl. XXIV, Fig. 8). Midway between two bundles is often a small group of phloem, unaccompanied by xylem, apparently formed by subdivision of one or two cells immediately within the starch-sheath. These represent the downward continuations of small bundles which, higher up, include xylem.

The course of the bundles is briefly as follows: Three bundles, each accompanied by a resin duct, enter the stem from each leaf, their downward path at first marked by green lines on the grey-green surface of the stem. The median bundle traverses the cortex within the space of five internodes or less. The smaller laterals diverge rather widely from it and enter the bundle ring at lower levels, not necessarily at the same level as each other. When all three have entered the ring, each lateral is normally separated by three other bundles from the median bundle. Commonly there are thirty bundles in the ring, larger alternating with smaller with some regularity. The median bundle enters the bundle ring just to the left (looking downwards from outside) of that of the next leaf below on the same orthostichy. The laterals enter in corresponding positions. The bundles thus appear commonly to remain individually recognizable for at least ten internodes; though they do not necessarily remain wholly free from other strands coming from higher up. As the stem grows, certain bundles become stronger and stand out, in a cleared preparation of the stem, as strands running for considerable lengths, occasionally anastomosing with similar strands. These strands simulate a cauline system. In reality they correspond with those strands in the Sunflower which remain in communication with a succession of new leaves at the apex and show a continuation of vigorous secondary growth. In *Kleimia*, as in the Sunflower, at any particular level of section, the trace-bundles entering from leaves close

above are the earliest to mature, but later they appear relatively small, owing to the greater subsequent growth of others, which receive continual accessions of strength from higher up the stem (11).

Certain deviations from the structures described above require brief record.

*Articulation.* (Text-fig. 3.) The articulation between a branch and its parent stem is usually very constricted. As the bundles of the branch approach the constriction they close in and anastomose, and all become strongly lignified. Nearer, the medullary rays become lignified so that a complete ring of lignified tissue is formed. It comprises short tracheidal elements as well as vessels, and lignified cells with conspicuously pitted walls. Sometimes the pith also is lignified, so that a solid woody core is formed. Lignification spreads upwards somewhat as the branch grows. From the constriction lignified tissue also spreads for a short distance in the bundle zone of the parent stem around the base of the branch. The union is not very strong and the branch is rather easily detached, coming away with the circle of radiating woody strands pulled out of the parent stem.



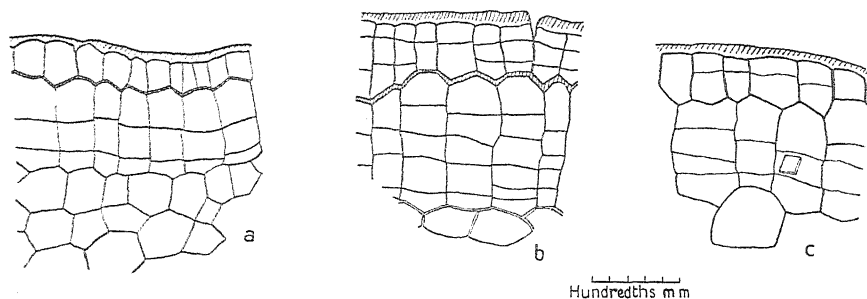
TEXT-FIG. 3. Longitudinal section through articulation between branch and parent axis. Lignified tissues cross-hatched except for strands of primary xylem seen running through. *c*, cells containing crystals of calcium oxalate.

In the epidermis at the articulation a phellogen arises which forms a periderm of six or seven layers. In the pith immediately below the constriction the closely packed, lozenge-shaped cells contain crystals (Text-fig. 3, *c*), mostly small clusters, some rhombohedral (probably calcium oxalate) but occasionally long needles. Similar crystals occur above the constriction in cells adjoining the lignified pith, and close examination reveals them in the lignified pith-cells as well.

*Rhizome.* (Text-fig. 1, *d, e, h, j*.) The structure varies in different rhizomes and in different parts of the same rhizome, largely according to the diameter. Where the diameter is large the structure in transverse section is very like that of the aerial stem (Text-fig. 1, *e*). Even then, however, the vascular tissue is more strongly developed: the bundles are rather large, there are more vessels, and cambial activity commonly extends across the medullary rays. On the other hand, the scale-leaves are small and their trace-bundles are very slender compared with those of the aerial leaves.

In slender, older rhizomes lignification follows cambial activity and

a complete ring of lignified tissue may result (Text-fig. 1, *h*). It is interesting that, here again, crystals of calcium oxalate are found in the parenchymatous (perimedullary) cells adjoining the lignified zone. With increasing diameter the zone tends to break up into several portions



TEXT-FIG. 4. Transverse sections of periderm of rhizomes. *a*, with hypodermal phellogen; *b* and *c*, with epidermal and hypodermal phellogens. In *c* one of the cells of the hypodermal periderm contains a crystal of calcium oxalate. Cutinized and suberized walls shaded.

(Text-fig. 1, *d*, *f*), or into individual bundles from which lignification only spreads a little way into the medullary rays. The thickest rhizome with a continuous woody ring had a diameter of 2.8 mm. A factor to be remembered in relation to this greater development of secondary tissues in the rhizome is the production of roots at intervals. Resin canals are hardly recognizable in the slenderer rhizomes; while they may be sometimes well developed in large rhizomes, more usually the ducts are narrow and without a definite epithelium. With regard to the occurrence of a Casparian strip and of a starch-sheath we can at present make no general statement. We have found a starch-sheath in some rhizomes, a Casparian strip quite continuous in others.

Periderm formation (Text-fig. 4), at first localized in longitudinal streaks, begins early. The phellogen is usually at first epidermal: but under cracks in the cuticle this tends to be superseded by a hypodermal phellogen. Even when the periderm reaches many layers in thickness, the boundary between epidermal and hypodermal periderm, i.e. the original inner wall of the epidermis, is often conspicuous as the only wall that has been suberized. After the phellogen has formed, several layers of cortical cells just below it become collenchymatous; later still calcium oxalate crystals appear in them. These features are not found in the rhizome before periderm formation is well advanced.

A conspicuous feature in spirit material is the large amount of inulin generally present, usually massed around each vascular bundle in the larger rhizomes.

*Peduncle.* The slender leafless stalk of the inflorescence is in transverse section very similar to a young aerial stem, perhaps with the bundles



rather closer together. Growth in length is very rapid. The epidermal cells are six to eight times longer than broad; the stomata are about eighteen to the square millimetre. The hypodermis becomes collenchymatous but forms no calcium oxalate. The mature fruiting axis (Text-fig. 1, *f, g*) shows a general lignification of the parenchymatous cells of the bundle zone. Not only are two or three layers of cells of the interfascicular parenchyma lignified, but also xylem parenchyma and small groups of cells outside the phloem of the principal bundles. The stalk, however, withers after flowering.

*Etiolated stems.* The etiolated stems, grown in complete darkness, are still slenderer, only 1.5 to 2.0 mm. in diameter. Stems grown in very dull light usually are quite as slender, though green. In transverse section (Text-fig. 1, *c*) they are like young stems on a smaller scale with fewer bundles. A Casparian strip occurs, but it shows some irregularities. Where it can be traced outside a bundle it traverses the inner cells of the sheathing layer of the resin canal. That is, it runs between the resin canal and the phloem, whereas the starch-sheath, as in the aerial stem, follows right round the resin canal on the outside. These are features which call for further investigation in order, if possible, to discover the factors on which their differentiation depends (compare the work of Bond (2) on the endodermis of *Piper*).

*Leaf.* The lamina, though flat, is distinctly succulent. The cuticle is thin, like that of the young stem, with a thin granular covering of wax. Stomata occur only on the lower side, averaging about 140 per sq. mm. between the veins. The contents of the guard-cells turn brown in iodine. The upper mesophyll forms a water-tissue of palisade-like cells, with scattered chloroplasts. It grades rapidly into a lower mesophyll of small cells with abundant chloroplasts. The whole of the mesophyll, including the water-tissue, is well provided with air-spaces. Taking the position of the vascular bundles as a dividing line it is interesting to find that the water-tissue is proportionately thicker in the young leaf, as is illustrated by the following measurements:

	Young leaf. mm.	Mature leaf. mm.
Water-tissue . . .	0.16	0.29
Assimilating tissue . . .	0.07	0.24
Total thickness . . .	0.23	0.53

The osmotic pressure of the sap is lower in the water-tissue, e.g. 0.4 M sucrose plasmolysed 50 per cent. of the cells of this tissue when 0.5 M sucrose was required to plasmolyse 50 per cent. of the cells of the assimilating tissue.

When the leaf begins to wither, an early symptom is a pitting of the upper surface due to local collapse of the water-tissue. How this tissue

behaves during temporary drought has not yet been determined: the leaf is thin and very delicate and therefore does not lend itself readily to the investigation.

Secretory canals traverse the main veins close to the phloem of the vascular bundles. Fusion reduces the number of bundles and canals to five at the base of the blade, and three a short way down the petiole.

The structure of the petiole is very simple: an epidermis with about thirty-five stomata per sq. mm. encloses parenchyma, some of the cells of which contain chloroplasts, and three bundles. On the adaxial side of the leaf-base is a small median patch of caducous hairs. Apart from these hairs the shoot is glabrous, as in most succulents.

The stem continues to grow after the leaf has reached maturity. Accommodation of the leaf-base to this growth is connected with the development of a phellogen across the leaf-base and in the epidermis of the stem for an irregular distance around. This eventually forms a periderm of five or six layers of cork-cells. Meanwhile the cells of the leaf-base above (outside) the periderm enlarge and some of them divide. Later they become lignified and lose their contents. During its formation this pad of tissue allows of expansion with the growth of the stem. There is no absciss layer: the leaves wither and remain hanging for some time still firmly attached.

Below the periderm a layer of crystal-containing collenchymatous cells is formed continuous with that below the epidermis of the stem. This correlation between cuticle, or cork, and collenchyma with calcium oxalate, has been the subject of a special investigation.

*Root.* The roots vary from diarch to pentarch. Root-hairs are found extending from near the root-tip back for several centimetres. There is a well-marked exodermis. The phellogen arises in the layer of cells next below (cf. van Tieghem (12), p. 719). In old roots, as elsewhere in the plant, a collenchymatous layer with calcium oxalate crystals is found immediately below the phellogen.

The endodermis, as is usual in roots, is marked by a Casparian strip. Just outside it resinous substances are found in intercellular spaces (cf. Solereder (10), p. 461, Fig. 104; Col (4)), but there are none of the highly differentiated ducts characteristic of the aerial shoot.

#### SUMMARY.

A general account of the morphology and anatomy of *Kleinia articulata* has been given.

The following points may be selected for special mention:

1. The length of the stem-joints varies greatly according to the cultural conditions.

2. Rhizomes are produced, which vary in form and diameter. Their anatomy shows a correlation between small diameter and a continuous ring of xylem on the one hand, and between large diameter and separate bundles on the other; but even in the thick rhizomes more vascular tissue is developed than in the aerial shoot, and there is more interfascicular meristematic activity.

3. The narrow articulation between branch and parent stem also shows strong lignification; and a ring of lignified tissue develops in the inflorescence axis.

4. A Casparian strip has been found in aerial stems between the phloem of the principal bundles and the resin canal which invariably accompanies them. In some rhizomes and in etiolated stems the endodermis, as defined by the presence of a Casparian strip, is complete. The starch-sheath, when present, is not entirely coincident with this, for at the bundles it follows the outside of the resin canals.

5. Below the epidermis in the aerial stem, and also below the periderm wherever it occurs, a layer or two of cells develop collenchymatous thickening, and in them rhombohedral crystals of calcium oxalate form. Elsewhere calcium oxalate has been found only in association with strong lignification, viz. in and around the lignified pith of the constricted articulation of a branch, and in the neighbourhood of the woody zone of slender rhizomes.

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# EXPLANATION OF PLATE XXIV.

Illustrating Professor Thoday and Mr. N. Woodhead's paper on A Preliminary Survey of the Morphology and Anatomy of *Kleinia articulata*, Haw.

Fig. 1. Plant in growing season, March, 1926, showing tapering apex, petiolate leaves, constricted articulation at base of branch, and slighter constriction after abortion of inflorescence.  $\times$  ca.  $1/5$ .

Fig. 2. Plant at end of growing season with tips rounding off and leaves withering.  $\times$  ca.  $1/4$ .

Fig. 3. Plant at end of growing season, April, 1931, grown with full mineral nutrition from cuttings planted October, 1930, showing young rhizomes growing obliquely downwards and swollen, rooted base. The lower rhizome is somewhat swollen, and the upper appears to be developing as a slender rhizome.  $\times$  ca.  $1/5$ .

Fig. 4. Vigorous plants, March, 1931, grown in sand-culture with full mineral nutrition from cuttings planted October, 1930.  $\times$  ca.  $1/10$ .

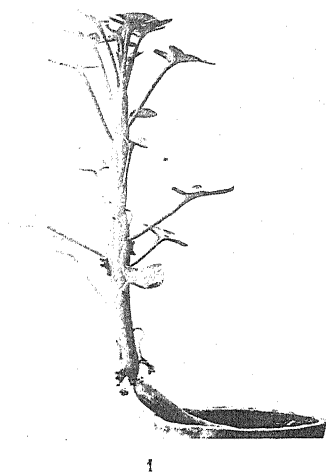
Fig. 5. Plants from cuttings planted October, 1930, one flowering, December, 1930.  $\times$  ca.  $1/10$ .

Fig. 6. Etiolated shoots grown in continuous, weak, artificial light for three weeks.  $\times$  ca.  $1/9$ .

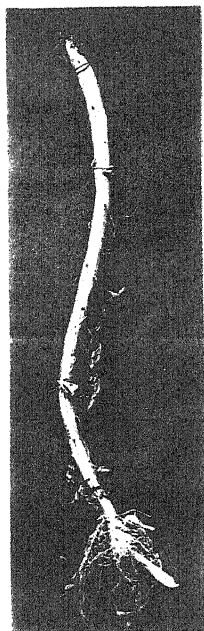
Fig. 7. Part of transverse section of aerial stem showing collenchyma, starch-sheath, vascular bundles widely spaced.  $\times$  30.7.

Fig. 8. Part of transverse section of older aerial stem showing some interfascicular meristematic activity immediately within the starch-sheath.  $\times$  30.7.

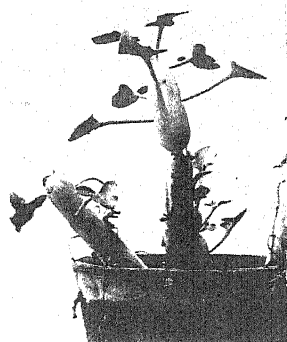
Fig. 9. Superficial tissues of aerial stem in transverse section showing thick cuticle and calcium oxalate crystals in hypodermal collenchyma.  $\times$  108.



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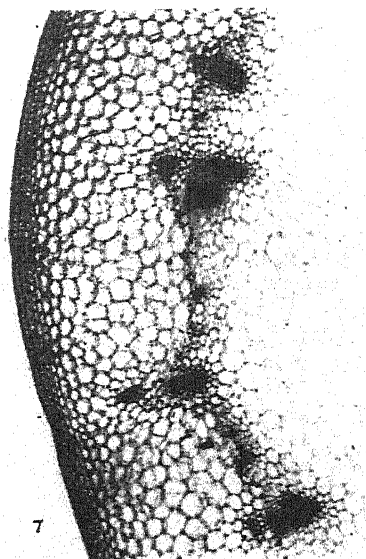
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# Polarity and the Production of Adventitious Growing Points in *Marchantia polymorpha*.<sup>1</sup>

BY

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With Plate XXV and thirty-one Figures in the Text.

## I. INTRODUCTION.

FÖRSTER (3), in 1926, studied the effect of external factors on the development of *Marchantia polymorpha*. He found that adventitious 'buds' were produced when plants were grown in a dry atmosphere, or on a medium containing 2.4 per cent. agar, and that this was also the case when grown on a concentrated liquid culture solution, and on plasmolysing with a strong solution of  $\text{KNO}_3$  (3–5 M.) and glucose (11–16 M.).

Vöchting (6), in 1885, studied the production of adventitious growing points from cut-off portions of the thallus and reproductive structures of *Marchantia*.

During experiments on the effect of X-rays on various plants the author found that the gemmae of *M. polymorpha* produced adventitious growing points or buds as a result of irradiation. This observation led to the following experiments in which the effect of various treatments on the production of adventitious growing points has been studied. Some of Förster's and Vöchting's experiments have been repeated and others performed along similar lines.

## II. METHOD OF CULTURE.

The gemmae were grown in a culture medium of the following composition:  $\text{CaH}_2(\text{PO}_4)_2$  0.1 gm.,  $\text{KNO}_3$  0.81 gm.,  $\text{MgSO}_4$  0.26 gm.,  $\text{FeCl}_3$  a trace, agar 15 gm., water 1,000 c.c. The medium was sterilized and poured into sterile Petri dishes, the gemmae being placed in rows on the surface at a distance of approximately 1 cm. from one another. Care was

<sup>1</sup> Part of thesis approved for the Ph.D. Degree of the University of London.

taken to ensure that the gemmae lay flat on the surface and were not partly immersed in the gel. The dishes containing the gemmae were placed under 100-watt lamps, and the heat from the latter was absorbed by a water-screen, so that the gemmae were grown at a temperature which remained reasonably constant at about 24° C. When it was necessary to compare the effects of different treatments under exactly comparable cultural conditions, the treated gemmae were kept separate in different sectors of the same dish.

### III. NORMAL GROWTH CHARACTERISTICS.

If the growth of a gemma is followed from the time of removal from the cup and being placed under favourable conditions for growth, it is seen that the cells of the general body of the gemma increase in size, and the cell number increases by division of the two apical cells. After seven or ten days' growth a new apical cell differentiates beside the original one at each growing point, according to the account given by Goebel (4), and thus is initiated the characteristic dichotomous branching, so that in from ten to fifteen days from the date of planting each gemma generally has four distinct growing points. Text-figs. 1-3 illustrate diagrammatically the normal course of development. Each apex is marked with a cross; and in Text-fig. 3 and at one end of Text-fig. 2 dichotomous branching has been initiated. This is the typical mode of growth, and the great majority of gemmae follow it in detail. Occasionally one of the apices fails to grow or grows more slowly than the second (Pl. XXV, Fig. 1), but irregularities such as these are rare. If the light intensity is reduced, branching of the thallus does not occur, or at most very infrequently.

An abnormal type of growth occasionally appears and has been observed in about 20 gemmae. In these cases the gemma develops nodules on the surface of the thallus (Pl. XXV, Fig. 2); these are irregular in shape and may occupy the whole of the surface or only a small region. The affected plant increases little in size, the nodules remaining quiescent for a period ranging from fourteen days to a month, when they develop short filaments which are very similar to those found in the air chambers, and which cover the entire surface of the affected parts. These filaments eventually divide longitudinally and form small thalli, each with an apical growing point. The thalli continue to increase in size and grow in the normal manner. As many as 150 such adventitious thalli have been counted arising from one gemma.

Occasionally, on the upper surfaces of the normal thalli, short filaments grow out from the epidermal cells, but these have not been seen to develop further, except in one case where the thallus was tilted so that the filaments came into contact with the agar gel, when they continued to grow in length



and later divided longitudinally producing apical growing points, and eventually forming normal thalli. This possibly forms an analogous case to that reported by Förster (3) which he figures on p. 371.

#### IV. EXPERIMENTAL DETAILS AND RESULTS.

##### 1. *The Effect of Treatment with X-rays.*

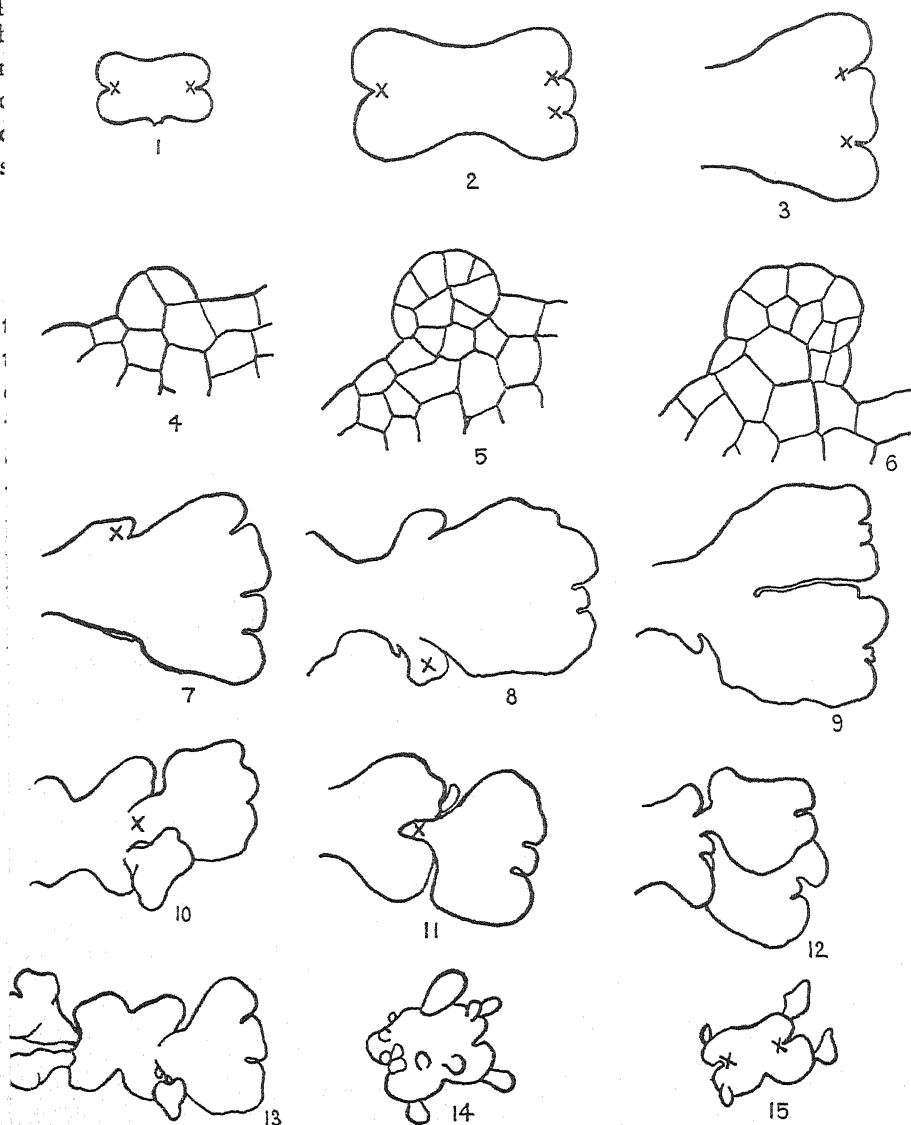
Gemmae were removed from a number of cups and placed in a tube, where they were well shaken with water. This suspension of gemmae was poured over a sheet of filter paper in a Petri dish and the plants evenly distributed over the surface. The dish was marked out into nine equal sectors by strips of paper laid on the surface, one sector, the control, was covered with a lead screen, and the dish then exposed to the X-rays.

The X-ray tube used was of the Coolidge pattern, and air-cooled. The gemmae were situated 18 cm. from the target, no screen being employed. The effective voltage used was 70 kilovolts and the current through the tube 3.5 milliamps. The sectors were irradiated for periods of 0.25, 0.5, 0.75, 1.25, 2.0, 2.75, 3.25, and 4 hours respectively; at the end of each period the appropriate sector being covered with a lead screen. A thermometer placed in the position occupied by the gemmae during irradiation registered a maximum temperature of 24° C., and on the bulb being covered with wet cotton-wool, 18° C. The filter paper on which the gemmae lay was kept moist during the experiment.

Immediately after irradiation the gemmae were placed on the culture medium in the Petri dishes. Each dish was divided into eight sectors, gemmae receiving the different 'doses' being placed in separate sectors. The gemmae were allowed to grow for ten days, when the number of adventitious buds was counted. Pl. XXV, Fig. 6, shows one of the dishes after such a period of incubation. The total number of gemmae irradiated in this experiment was 3,466 with an average of 433 gemmae for each of the eight periods of exposure. Over 300 non-irradiated gemmae were grown as controls.

Irregularly shaped lobes sometimes appeared on the thallus as a result of irradiation, and the same criterion was used in distinguishing these from true adventitious growing points as that employed by Förster in his experiments with plasmolysing solutions. Those composed of large-celled tissue, without apical growing points and only attaining a limited size, are not considered true adventitious buds. True buds are formed of small-celled embryonic tissue with an apical growing point, though in very young buds the latter cannot be distinguished. The earliest stage in the production of adventitious buds which has been observed consisted of a group of cells of a smaller size than the surrounding ones rising above

the general level of the thallus (Text-figs. 4-6). These gradually increased in size and became flattened, when an apical growing point appeared



TEXT-FIGS. 1-15. For explanation see text.

(Pl. XXV, Figs. 3, 4, 5). These adventitious thalli have been detached and grown for nine months on soil in a greenhouse, during which time they have behaved normally in every respect.

The development of the normal apices of the gemmae is markedly

affected by X-radiation. Though the variation between individuals is considerable, the general effect as the 'dose' increased was as follows. The first effect to be observed was the formation of 'shoulders' of tissue (Text-fig. 7), which appeared on each side of the gemma behind the apical area. As the dose increased these shoulders of tissue became relatively larger, so that the tissue joining the apical area to the older parts of the gemma became narrower (Text-figs. 10 and 11), and at the same time adventitious buds arose between the lobes and the apical area (Text-figs. 8, 10-13) and occasionally at other positions near the base of the apical zone. Sometimes the apical area consisted of two approximately equal branches (Text-fig. 9). As the periods of exposure increased still further the apical area decreased in size and eventually did not develop (Text-figs. 14 and 15). Also, at the longer periods of irradiation the adventitious buds, besides being more numerous, were scattered over the surface of the gemma, though they were still most plentiful in the neighbourhood of the apices (Text-fig. 14). They arose on both the upper and lower surfaces of the gemma, but were most numerous on the upper. After an exposure of four hours it was found that the cells in the vicinity of the apical cells were killed (Text-fig. 15). The general effect of the rays was to reduce the growth of the thallus, so that after ten days' growth gemmae exposed for three to four hours showed only a small increase in area.

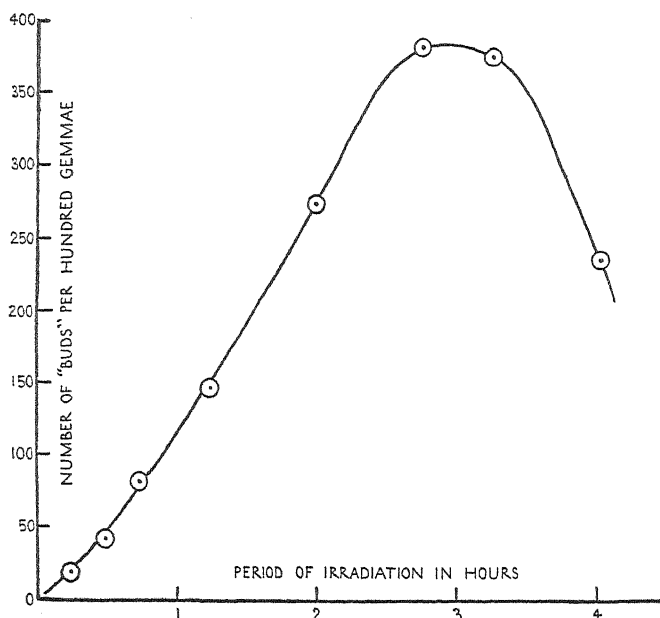
Table I shows the mean number of adventitious buds produced per hundred gemmae for each of the eight different periods of irradiation. Text-fig. 16 illustrates graphically this relationship, and it can be seen that the rate of production of buds at first increases, but later falls off rapidly, owing to the lethal effect of the rays.

TABLE I.

Period of Irradiation in hours.	Mean number of buds per hundred gemmae.	P.
0.0	0.0	—
0.25	19.1	0.4
0.50	40.5	0.1
0.75	83.0	0.1
1.25	147.0	0.01
2.00	272.1	—
2.75	380.3	—
3.25	376.1	—
4.00	233.5	—

The distribution of the buds among the gemmae at each period of irradiation up to 1.25 hours followed a Poisson series, but above this dose there were too many low values of bud numbers, and also the relative numbers of gemmae with many buds was too great. The value of  $P$ . for each period of exposure up to 1.25 hours, corresponding to the value of  $x^2$

obtained, is given in Table I. These values were taken from the table of  $\chi^2$  given by Fisher (2), and it can be seen that the agreement with the Poisson series is for the first three values reasonably good.



TEXT-FIG. 16. The number of buds produced by different periods of exposure to X-rays.  
Curve of closest fit.

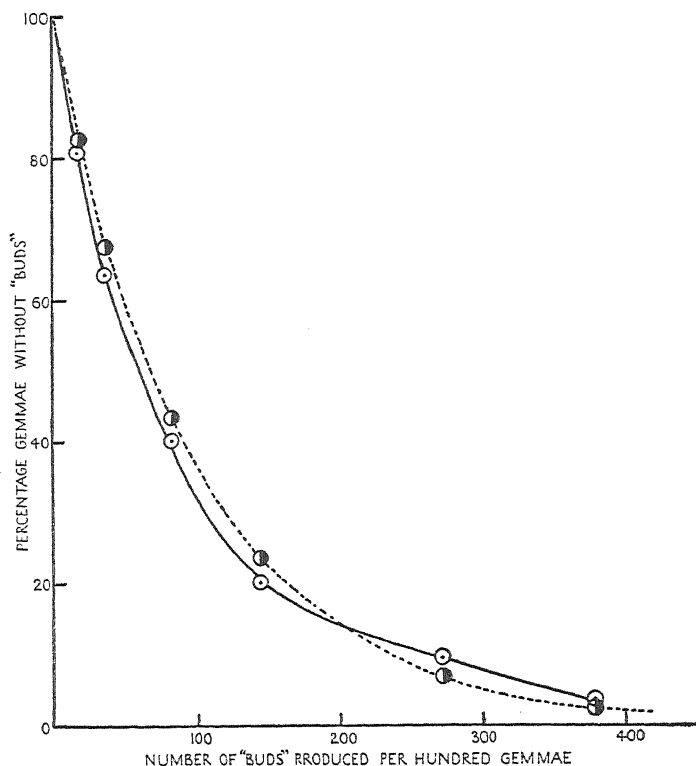
Table II contains the percentage numbers of gemmae for each period of irradiation with bud numbers up to three. The calculated values were obtained by taking the average number of buds per gemma for each period of irradiation as the mean of a Poisson series.

TABLE II.

Irradiation in hours.	% numbers of gemmae with various 'bud numbers'.							
	Found.				Calculated.			
	0.	1.	2.	3.	0.	1.	2.	3.
0.25	80.6	19.3	0	0	82.6	15.9	1.5	0.09
0.50	63.6	32.0	4.22	0	66.7	27.0	5.5	0.74
0.75	40.1	40.4	16.2	3.27	43.6	36.2	15.0	4.15
1.25	20.0	36.5	26.6	11.9	23.0	33.8	24.7	12.1
2.00	9.5	26.8	21.7	15.8	6.6	18.0	24.5	22.2
2.75	3.2	13.9	18.5	18.0	2.24	8.5	16.1	20.4
3.25	4.1	11.3	22.0	17.0	2.32	8.7	16.4	20.6
4.00	14.3	24.3	21.1	20.2	9.68	22.6	26.4	20.5

Up to 1.25 hours' irradiation the agreement is reasonably good, but above this the numbers of gemmae with 0, 1, and 2 buds is relatively too

high, though the discrepancy decreases with increasing numbers of buds. Text-fig. 17 shows the relation between the number of gemmae without buds and the average number of buds produced as the dose is increased,



TEXT-FIG. 17. The number of gemmae without buds plotted against the mean number of buds produced as the period of exposure to X-rays was increased. The continuous line represents the frequencies found, the dotted line the calculated values.

and illustrates the divergence referred to above, since the observed values are lower than the calculated values for the first part of the curve, but later exceed them.

To determine whether intermittent irradiation produced a response different from that due to continuous treatment, a number of gemmae were evenly distributed over a sheet of moist filter paper contained in a Petri dish, and were divided into two equal groups. The entire plate was irradiated for 2 hours, and 20 minutes later one group was irradiated for a further 2 hours, the second being shielded from the rays by a lead screen. The dish was then left at room temperature in a moderately strong light for 48 hours, when the second group was irradiated for 2 hours, the first being shielded. In this way each group received two periods of irradiation each of 2 hours' duration, one group having a 20-minute interval and the

second a 48-hour interval between successive periods. The gemmae were then placed in five Petri dishes containing culture medium, some 50 gemmae from each group being placed in the same dish. They were allowed to grow for 20 days and were then examined, the area of the thallus being taken as a measure of the effect of irradiation.

The gemmae in each of the five plates in which the radiation had been intermitted for a period of 48 hours were definitely smaller than those which had received almost continuous irradiation. The size of the individual gemmae varied greatly and some were killed in both groups, but despite this the difference between the groups was very marked, as is shown in Pl. XXV, Fig. 7.

The experiment was repeated on two occasions. In the first of these no difference was observed between the groups in each of four plates, whereas on repeating the experiment for the second time the same effect as in the original experiment was obtained in all three plates.

The results of these three experiments show that intermittently irradiated gemmae in eight plates were smaller than those which had been irradiated continuously; in four plates no difference was discernible.

### *2. The Effect of Variations in the Culture Medium.*

The gemmae were grown on media modified in various ways; two plates, each containing 100 gemmae, were used with each of the different treatments.

The pH was altered by the addition of varying amounts of HCl and KOH to the medium. An increase of pH produced no effect, but on decreasing it to 4.4, below which value the gemmae were killed, a few adventitious buds were formed on several gemmae and the apices grew out as with the X-ray treatment.

The gemmae were next grown on filter papers moistened with solutions lacking in Ca, K,  $\text{NO}_3$ , and  $\text{PO}_4$  respectively. The absence of Ca and K had little effect, whereas when  $\text{NO}_3$  and  $\text{PO}_4$  were lacking growth practically stopped, but in no case were adventitious buds formed.

The concentration of the mineral salts of the agar gel was increased ten times without producing any apparent effect.

The amounts of  $\text{CaH}_2(\text{PO}_4)_2$  and of  $\text{KNO}_3$  in the gel were then increased respectively to twenty times the normal amount. In both cases a reduction in size with increasing concentration and some irregularity in the shape of the gemmae were observed, but no adventitious buds were produced.

### *3. The Effect of Drying.*

The gemmae were exposed for various periods over different strengths of  $\text{CaCl}_2$  solutions. No effect was produced beyond a reduction in size

even when the atmosphere was only 60 per cent. saturated at 20° C. One hundred gemmae were next placed in a desiccator for varying periods up to sixty minutes. The size was reduced with increasing time of exposure and a number of gemmae were killed. Twelve gemmae produced adventitious growing points; some of these had been exposed for ten minutes in the desiccator, the others for a longer period.

#### 4. *Temperature Effects.*

The gemmae were exposed for various periods to temperatures ranging from 4° C. to 35° C. while kept in a saturated atmosphere. No adventitious buds were formed.

#### 5. *The Effect of Toxic Agents.*

Gemmae were placed in atmospheres containing absolute alcohol, ether, and chloroform respectively for varying periods. Killing, reduction in size, and irregularity in shape resulted, but no adventitious buds were produced. A further number of gemmae was then immersed in dilute solutions of iodine and bromine respectively; these showed a reduction in size and many were killed. In some plants, however, groups of green cells were surrounded by dead brown ones, and the former grew out to form adventitious growing points.

#### 6. *The Effect of Weak Doses of Ultra-violet Radiation.*

The effect produced by exposure to ultra-violet radiation was similar to that from treatment with iodine and bromine. The normal apices grew out to produce new thalli, and adventitious buds were formed from groups of green cells scattered over the surface of the gemma, the rest of the tissue being brown and dead.

#### 7. *The Effect of Plasmolysis.*

The gemmae were immersed in 1 M.  $\text{KNO}_3$  (vol. normal) for varying periods up to twenty-four hours; they were then washed in water for one hour and placed on culture medium. They showed a decrease in size with increasing periods of immersion, and a high percentage was killed. Some of the survivors produced adventitious growing points, which arose from all points of the surface of the smaller plants, but in the larger ones they were generally restricted to that portion of the gemma midway between the apices, and in the latter case were less numerous.

A similar experiment was carried out using a 5 M. (vol. normal) solution of glucose in place of  $\text{KNO}_3$ . The gemmae were immersed in the solution for six hours. The effect was very similar to that obtained with  $\text{KNO}_3$ . Various strengths of sucrose and  $\text{KNO}_3$  up to 1 M. (vol. normal)

were next used, the period of immersion in this case being two hours. These produced effects similar to the preceding experiments, but the number of buds produced per gemma fell off rapidly with decreasing concentrations. The production of buds was, as in the former cases, very irregular, some gemmae having a large number, while others, which had received exactly similar treatment, produced them very sparingly or not at all. Adventitious buds appeared in a few gemmae exposed to 15 per cent. M. solutions of each substance.

#### 8. *The Effect of removing the Apices and dividing the Gemmae in Various Ways.*

One of the apical cells and a small area of tissue adjacent to it were removed from each gemma with a fine needle, and the plants left to grow for some days, when adventitious buds were found to have arisen in the region of the removed apex.

In a second experiment both apices and the small areas of tissue adjacent to them were removed. Adventitious budding was much more plentiful in this case, the buds arising from all points of the surface, though most plentifully at the apical ends. Considering the number of buds produced, the plants grew more strongly than was the case with those which had been X-rayed, and were very similar to some which had been plasmolysed. As was to be expected from the point of view of nutrition, the adventitious buds grew more rapidly when few in number than when large numbers were produced on individual gemmae. This experiment proves that, either directly or indirectly, the apical region inhibits the production of adventitious growing points.

In the following series of experiments the gemmae were grown for seven to ten days, by which time they were about 0.5 cm. long, and were then divided with a razor as described below, after which they were replaced on the culture medium.

The various methods of treatment are shown diagrammatically in Text-figs. 18–31. Text-fig. 18 represents the gemma before treatment. The dotted lines in the other figures represent the direction of the cuts in each case; where these are not continuous across the gemma it signifies that the portions were left connected where the line does not run.

No adventitious buds were produced when gemmae were cut transversely into two parts, as in Text-fig. 19. When, however, the cut was not central, as in Text-fig. 20, buds were produced from the cut edge (marked x) in all cases, but never from the cut-off apex. The same effect was produced on treating the plants as shown in Text-fig. 21, buds being produced at the cut edges (indicated with a x). In Text-fig. 22 buds were produced at the parts of each segment nearest the apex.



In the last three experiments the adventitious buds arose in most cases from the middle of the cut edge.

In gemmae treated as in Text-fig. 23 adventitious buds were produced at the apical ends of each of the four outside pieces, and occasionally at their bases; they were also more plentiful on the inner than on the outer edges of these pieces. In the experiment illustrated in Text-fig. 24 a similar response was observed, and buds were also produced from each of the four central pieces at the end nearer an apex.

In Text-figs. 25-9 inclusive, in which the parts were attached to one another by only a small strip of tissue, sheets of mica were inserted between the cut surfaces to prevent diffusion of substances across the gel from one part to the other, so that any inhibiting influence exerted by the apex had to be conducted through the small piece of tissue connecting the segments.

The response to the injury illustrated in Text-fig. 25 was not so definite as in the preceding experiments; in seven cases buds were produced at the cut surfaces (marked with a  $\times$ ) and in twenty half-gemmae no adventitious buds were formed. Three plants produced buds at the basal end.

Forty-four half-gemmae were treated as shown in Text-fig. 26, adventitious growing points being formed at the cut edges (marked A, B, and C). In Table III the + signs indicate the presence of buds, and the - signs their absence at the edges A, B, and C respectively. The figure in the first column indicates the number of plants with and without buds in these three positions.

TABLE III.

	A.	B.	C.
4	-	-	-
2	-	-	+
17	-	+	-
3	-	+	+
3	+	-	-
2	+	-	+
8	+	+	-
5	+	+	+

From this table it will be seen that the total number of plants producing buds at the edges A, B, and C was 18, 33, and 12 respectively, while the number which produced them at A but not at B in the same plant was 5, and at B but not at A was 20.

No adventitious buds were produced by the treatment illustrated in Text-fig. 27.

In thirteen out of fifteen half-gemmae buds were produced at the cut surface remote from the apex when the gemmae were cut as in Text-fig. 28, and buds occurred in a similar position in all cases on treatment as

shown in Text-fig. 29, and were larger than was the case with the previous treatment.

As shown in Text-fig. 30, two strips of thallus were cut from each of the lobes at the sides of the apical depression. The outer strip A, and the inner B, each produced adventitious buds at both ends, but more plentifully at the end next to the primary apex of the plant. The outer strip, A, also produced buds at the centre of the uncut edge, whereas the inner one, B, produced them on both cut edges, though more plentifully on the edge nearer to A.

In Text-fig. 31 the gemma is cut in two transversely, and the two apical parts removed, the remaining portions being cut into strips longitudinally. These strips were placed on the culture medium; eight had the ends which were originally nearer the primary apex and seven had the basal ends covered with black paper, thus preventing direct light from reaching the covered portions, though a small amount of diffused light could reach them through the agar.

In eight days all the exposed apical ends had produced adventitious buds, and in one of these strips the covered basal end formed them also. Six of the strips which had the apical ends covered developed adventitious growing points at the exposed basal ends, and four of these at the covered apical ends also. Two strips did not produce any buds. The black paper was then removed, and in a further seven days the latter group had produced buds at all the apical ends, though these were not so large as those at the bases, and the latter, in their turn, had not grown so rapidly as the buds from the apical ends originally exposed.

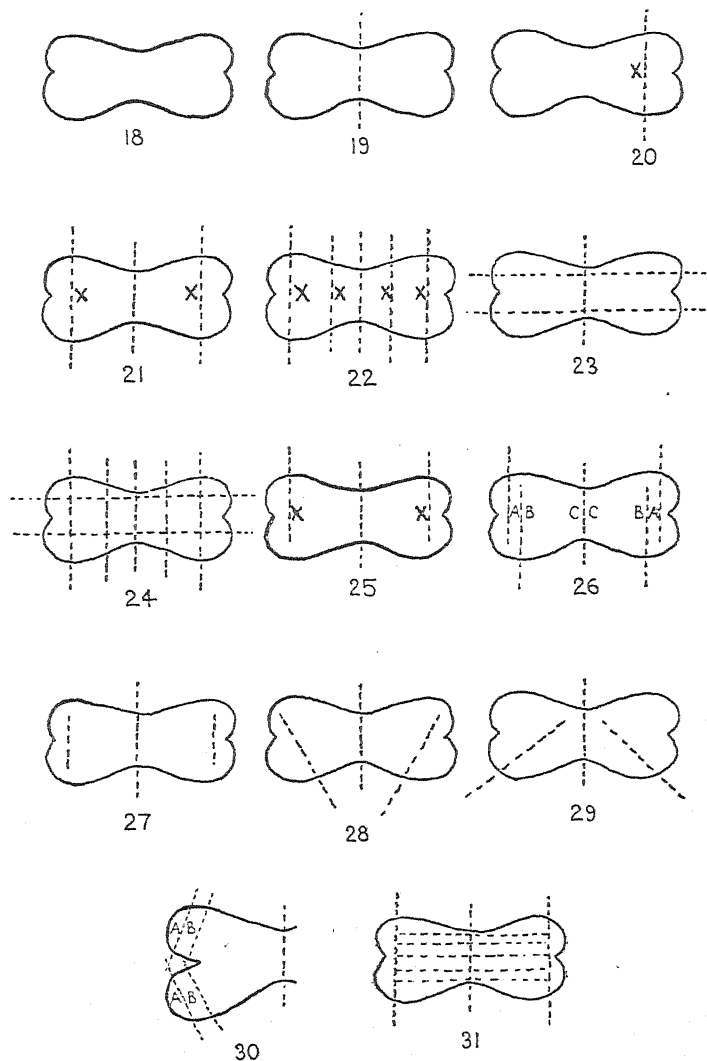
This experiment was repeated in a modified form. Twelve plants were taken and cut transversely in half, and the apical portions of each removed as in the former case. The remaining pieces were not, however, cut into strips but were placed entire on the agar medium. The apical parts of twelve of these and the basal parts of the other twelve were covered with black paper.

All the apical and two of the basal regions of the latter group produced adventitious growing points, while of the twelve pieces whose apical ends were covered, six produced adventitious buds at the exposed parts and six at the covered apical ends.

#### 9. *The Exposure to X-rays of Gemmae whose Apices were removed.*

Three hundred gemmae were taken and divided into three groups of a hundred each. The two apices and the small areas of tissue adjacent to them of each of the gemmae in the first and third groups were removed, and the plants of the first group were then irradiated for three hours. The gemmae of the second group were irradiated for a similar time, but were otherwise untreated. All the gemmae were grown for ten days, when the

number of adventitious buds was counted and the following results obtained.



TEXT-FIGS. 18-31. For explanation see text.

The figures below represent the numbers of buds per 100 gemmae treated.

	Mean.	S.E.
Gemmae irradiated after removal of the apical regions	417 % $\pm$ 22.7	
Apical regions removed	94 % $\pm$ 6.95	
Irradiated	275 % $\pm$ 15.9	

The experiment was repeated, 50 plants being treated in each group. In this case the gemmae were five days old before treatment.

The mean numbers of buds per 100 gemmae were as follows :

	Mean.	S.E.
Gemmae irradiated after removal of the apical regions	502 %	$\pm 38.4$
Apical regions removed	270 %	$\pm 18.6$
Irradiated	464 %	$\pm 27.7$

It is seen that irradiation has a much greater effect than removal of the apices and small areas of tissue adjacent to them in so far as bud production is concerned, and that irradiation and removal together produce a greater response than irradiation alone.

#### 10. *The Conduction of the Inhibitory Effect of the Apex through a Zone of Dead Cells.*

In order to ascertain whether the inhibitory effects of the apex could be transmitted through dead tissue a number of gemmae were strongly irradiated with ultra-violet rays, having previously been covered with black paper except for two narrow transverse strips near each apex. The black paper prevented the rays from reaching the covered parts, so that only the two exposed strips of each gemma were affected. The tissues thus exposed were killed.

After irradiation, the paper was removed and the plants left to grow for a fortnight. Upon examination, three plants were found to have produced adventitious buds at one killed edge on the side remote from the apex, six had developed them at both similar edges (Pl. XXV, Fig. 8), two had produced no adventitious buds, and two adventitious buds on the apical side of the killed strips. The killed tissue was flaccid and brown in colour, though still holding the living portions firmly together.

In all the experiments described, unless otherwise stated, adventitious buds, when produced, arose from the lower surface of the thallus.

### V. DISCUSSION.

The series of experiments in which the effect was observed of removing the apices and cutting the gemmae into pieces in various ways points clearly to the presence of two 'polarity' gradients in each gemma, as indicated by the production of adventitious growing points.

If the gemma is cut into two equal parts by a line at right angles to that joining the two apices (i.e. transversely), each apex is the dominant portion of the half to which it belongs. That the dominating influence of any one apex does not extend far beyond the region midway between the apices is shown by the production of adventitious buds at the cut edge of the larger piece when the gemma is divided transversely into two unequal

parts, as shown in Text-fig. 20, whereas no buds are formed when the cut edge is midway between the apices (Text-fig. 19).

If a gemma is assumed to be divided transversely into two equal parts, and a series of lines drawn parallel to the longitudinal axis of the gemma, then any point on one of these lines will be dominant (in so far as the production of adventitious buds is concerned) to any other point on the same line if the latter is farther from the line dividing the gemma transversely into halves. Also, of any two points on a line at right angles to the longitudinal axis that which is nearer this axis will be dominant to the other (see the results of the experiments figured in Text-figs. 21-4 and 30).

If the manner of growth of a gemma is considered, it will be seen that in any half of the gemma the dominance of one zone of tissue over another is dependent on the relative age of the two groups of cells concerned, the younger group being in all cases dominant over the older.

This relation of age to the production of buds is also brought out by the results of removing the apical cells, and those adjacent to them, since in this case, though the buds arise from all parts of the surface, they are most plentifully produced around the apical region.

That this dominance of younger cells over older ones can be affected by the environment is shown by the experiments illustrated in Text-fig. 31, and in the succeeding experiment, but whether the partial reversal of polarity was due to the reduction of photosynthetic activity in the covered portions of the tissue, or to a difference in temperature between the covered and exposed parts (owing to the absorption of heat by the paper, or to the heat resulting from the absorption of the light rays) has not been determined. Neilson Jones (5) found that by keeping the two ends of a sea-kale cutting at a difference of temperature of 2° C. the polarity was reversed, the region at the higher temperature becoming dominant, and it is possible that a similar thermal effect may be responsible for the change in polarity recorded in the present experiments.

As would be expected, the inhibiting action of the apex is markedly affected by distance. In the experiment illustrated in Text-fig. 26, though buds are produced at the edges A and B, the inhibiting effect of the apex is evidently still present at these points, as otherwise budding would have occurred in all the plants tested, and the number of times it occurred at A should have exceeded that at B, since the cells at A are younger than those at B, and the tissue between the cuts, if it produced any effect at all, would tend to inhibit the production of buds at B. In fact, however, buds appeared at A on eighteen, and at B on thirty-three occasions, thus indicating that the influence of the apex is marked at A and less marked at B. A similar decrease in the intensity of the inhibiting influence as the distance from the apex increases is apparent from the results of the

experiments illustrated in Text-figs. 28 and 29, since in the latter case the inhibiting influence has further to travel than in the former and the buds are produced in a slightly greater number of cases, and when formed grow more rapidly.

That the inhibiting influence is not conducted through a zone of dead cells is shown by the results of Experiment 10, in which adventitious buds arose from cells separated from the apex by a strip of tissue killed by exposure to ultra-violet radiation. This conclusion is also borne out by the results of the experiments in which entire gemmae were subjected either to a weak dose of ultra-violet radiation or to immersion in dilute solutions of iodine and bromine. The effects of these treatments were very similar, groups of cells being killed, and those which remained alive producing adventitious growing points.

*The effect of increasing the dose of X-rays.* With small doses the effect of the rays is to prevent the division of the older cells of the thallus which results in the production of the 'shoulders' of tissue shown at  $\times$  in Text-fig. 7. With greater doses this effect is extended to the younger cells, so that the 'shoulders' of tissue become larger and the tissue joining the apical area to the older parts of the gemma becomes correspondingly narrow ( $\times$  in Text-figs. 10 and 11). With the longest periods of irradiation the division of the apical cell itself is prevented and the tissue surrounding it is killed ( $\times$  in Text-fig. 15). The first effect of the rays, then, is to prevent cell-division, the older cells being more sensitive in this respect than the younger. The second effect to be observed, and which is seen as soon as the division of the youngest cells is prevented by prolonged irradiation, is the death of the younger cells. These effects would be explained if the action of the rays in causing the death of the cells first became apparent as a stoppage of cell-division, and if in addition it were assumed that the younger cells, although they are the more sensitive cells, being the first to be killed, do not show the first effect of the rays as soon as the older ones owing to the greater potentiality for division which they possess.

*Development of adventitious buds.* The production of adventitious buds in general follows closely the zone of tissue where cell-division has been retarded or stopped by the action of the rays, so that as the dose is increased from zero buds are produced nearer and nearer to the apex. After this stage, however, the buds are more scattered, though the numbers are still greatest near the apex. With the apical cells the dose reaches lethal level first, with the result that bud production is gradually confined to cells further removed from the apex.

Assuming a variation in the resistance to the rays of cells of approximately equal age, and that less resistant cells do not convey the inhibiting influence of the younger cells so easily as do the more resistant ones, it is possible to explain the production of adventitious buds as they occur. If,

for example, a few cells near the apex happen to be highly susceptible to the rays, then the inhibiting influence of the younger cells around the apex will not be conveyed as strongly to cells behind the highly susceptible ones, and cells thus shielded would be able to divide.

*Rate of bud production.* As the dose increases and the cells in close proximity to the apex become affected, the general inhibiting influence of the apical area on the rest of the thallus will decrease, and cells separated from the apex by highly susceptible ones, but which previously had not been sufficiently freed from the inhibiting influence to develop buds, would now be enabled to do so, with the result that the rate of bud production would be increased with increasing time of exposure. Finally, as the dose increased still further, the number of buds produced would fall off rapidly, owing to the death of the younger cells. These were the effects actually obtained.

The fact that the distribution of buds among the gemmae takes the form of a Poisson series, in so far as the shorter periods of exposure are concerned, is what would be expected from the hypothesis set out above, since the buds have a relatively small chance of occurring, are produced independently of one another, and are distributed at random among the gemmae.

*An alternative theory of bud production.* That budding may be the result of a stimulating effect of the rays is possible, and if this effect took the form of separate 'hits', for example, if a bud resulted from the ionization of some particular molecule (or of a molecule of some particular type) in the cell, and the chance of such a hit occurring were small, the numbers of gemmae which had received 0, 1, 2, &c. hits would take the form of a Poisson series. In *Colpidium colpoda* the suggestion that a number of discrete hits are necessary to cause death has been put forward by Crowther (1) as a possible interpretation of his results, and he finds that a dose somewhat less than that necessary to produce a lethal effect causes active division in that organism. If one hit produced one bud, the number of buds produced would be approximately proportional to the time of exposure (the ratio of the numbers of buds to cells being small so that the number of cells available for bud production is not soon reduced), but from experiment this was not the case, the rate of bud production increasing with time. It would, however, still be possible to account for the increase in rate if more than one hit per cell were required to produce one bud. Thus, if  $m$  is the mean number of hits per cell, and the distribution of the number of hits falls into a Poisson series, and two hits are required to produce a bud, then the chance of two or more hits occurring in a particular cell will be the sum of all except the first two terms of the series, i.e.  $e^{-m}\{e^m - (1 + m)\}$ , and this is the chance of a cell producing a bud in a given period of time. The mean number of buds produced by  $n$  cells would

therefore be  $ne^{-m}\{e^m - (1+m)\} = n\{1 - (1+m)e^{-m}\} = z$ , where  $z$  is a function of  $m$ .

$$\begin{aligned}\text{The rate of increase of } z &= \frac{dz}{dm} = -n\{e^{-m} - (m+1)e^{-m}\}, \\ &= ne^{-m}(m)nme^{-m}, \\ \text{and } \frac{d^2z}{dm^2} &= n(e^{-m} - me^{-m}), \\ &= ne^{-m}(1-m).\end{aligned}$$

The point of inflexion therefore occurs when  $m = 1$ .

When  $m < 1$  the slope increases, and when  $m > 1$  the slope decreases; with the result that so long as the mean number of hits per cell is less than one, the rate of bud production will increase.

The hypothesis that buds are due to discrete stimulating influences will therefore explain the distribution of the buds and the increasing rate of production of buds at the smaller doses.

## VI. SUMMARY.

An investigation on the effect of X-rays on gemmae of *M. polymorpha* led to an examination of polarity and dominance in these structures. Gemmae were cut into pieces in various ways, and from the position at which adventitious buds on the gemmae were produced it appears that the younger cells of any part of the thallus always dominate the older in so far as the production of buds is concerned.

The intensity of the inhibiting influence decreases as the distance from the apex is increased.

The dominance of the younger over the older cells is reversed if the former are shielded from the light.

The inhibiting influence of the younger cells is not transmitted across a zone of dead cells.

Gemmae were also caused to produce adventitious buds as a result of various types of treatment such as ultra-violet light, X-rays, drying, and plasmolysing solutions.

The relation between bud production and increasing doses of X-rays was studied on nearly 4,000 gemmae, and it was shown that at any one dose the numbers of gemmae having 0, 1, 2, 3, &c., buds took the form of a Poisson series.

Two possible hypotheses are put forward to explain the observed effect of X-rays; one is on a basis of the lethal effect of the rays and of the variation in resistance to the rays of cells of different age, and the other is dependent on the assumption that budding is the result of discrete hits on cells, which are thus stimulated to divide.



In conclusion I wish to acknowledge my indebtedness to Professor V. H. Blackman for his valuable help and constructive criticism.

I also have pleasure in thanking Dr. F. G. Gregory, and Dr. J. O. Irwin, for their kind help with the statistical side of the problem.

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# EXPLANATION OF PLATE XXV.

Illustrating Dr. Dickson's paper on Polarity and the Production of Adventitious Growing Points in *Marchantia polymorpha*.

Fig. 1. A normal plant after eight days' growth. A second apical cell has differentiated at one apex, and dichotomous branching is about to occur. The dark areas on the surface of the thallus are air-chambers.

Fig. 2. Abnormal nodules of tissue on the thallus which later produced adventitious growing points.

Figs. 3-5. The plants are shown ten days after treatment with X-rays. The air in the chambers has been removed.

Fig. 3. One apex has grown out to form new tissue which remains connected to the gemma by a narrow neck. Two adventitious buds have been produced.

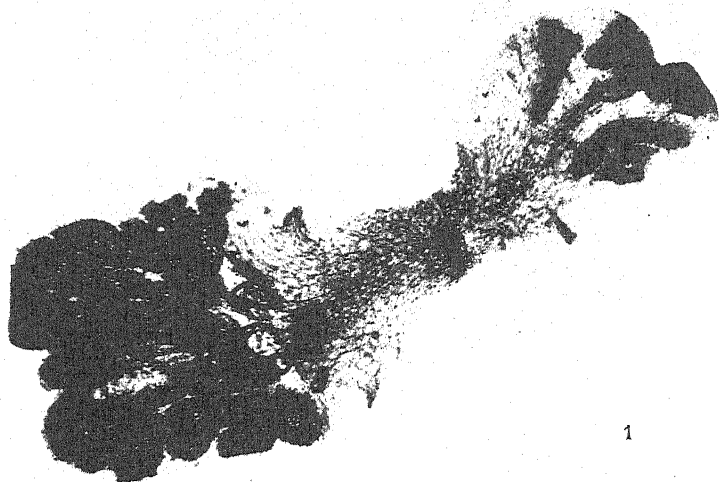
Fig. 4. Both apices have grown out to form new areas of tissue, and two adventitious buds have been formed.

Fig. 5. The two apices have grown out, and three adventitious buds have arisen as a result of X-radiation.

Fig. 6. Gemmae growing on culture-medium ten days after being X-rayed. The plants in the different sectors have been irradiated for periods ranging from  $\frac{1}{4}$  to 4 hours respectively. The reduction in size with increasing dose can be seen.

Fig. 7. The two sectors marked with a cross contain plants irradiated continuously for 4 hours; the plants in the other sectors were irradiated for 2 hours, and 48 hours later for a further 2 hours. The photograph was taken three weeks after the gemmae were irradiated, and, despite considerable variation in the size of the individuals, the plants which were irradiated intermittently were, on the whole, considerably smaller than those which had been treated continuously.

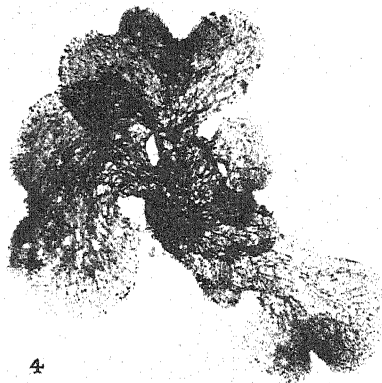
Fig. 8. A plant three weeks after two strips of tissue near the apices had been killed by exposure to ultra-violet light. The dead tissue is seen as two transverse bands of a lighter colour than the surrounding cells. Since being treated the apices have continued growing, and two adventitious buds have arisen from the edges of the dead zones on the sides removed from the apices. The plant was grown in weak light.



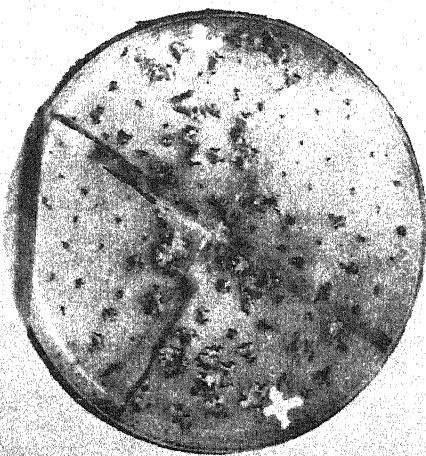
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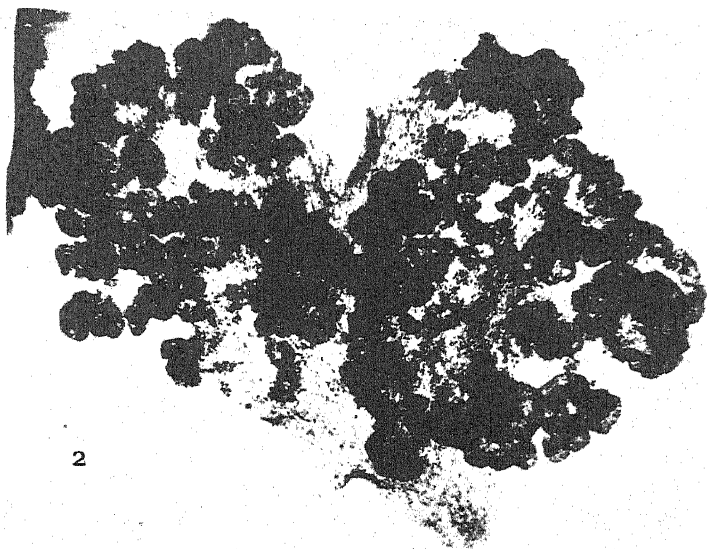
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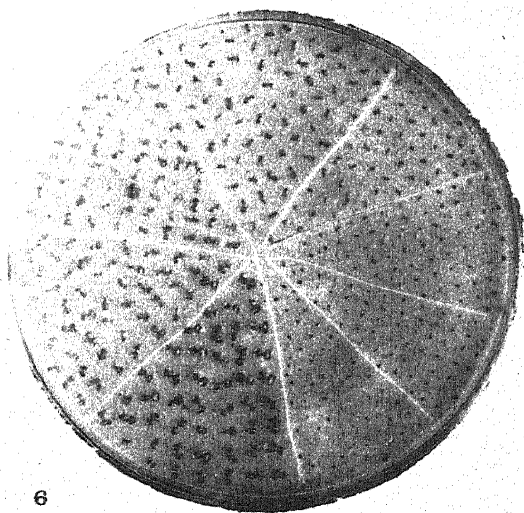
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6





# On the Mitotic Division of *Draparnaldia glomerata*.

BY

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With Plate XXVI.

THE writer hoped to be able to follow out the nuclear cycle of *Draparnaldia glomerata* (Bory) in full before publishing her observations. Unfortunately it is not at present possible to obtain supplies of material, as the alga disappeared from its habitat during building and other operations carried out in connexion with the Hathersage water supply, and has not yet become re-established. It is therefore thought desirable to record such facts as are available, and these relate only to the vegetative divisions. As far as the writer is aware, the only attempt to investigate this alga cytologically was made in 1915 by Hermann von Neuenstein (5), who was unable to obtain any data because of the small size of the nucleus and the difficulty of distinguishing it from the pyrenoids.

## MATERIAL AND METHODS.

Collections of *D. glomerata* were made regularly from October 1928 to August 1930, chiefly from a stream at Longshaw, near Sheffield, where the alga was always to be found growing on the gravel bed, and where it became very abundant during the summer months. Zoospores were usually produced the day after collection. Various fixatives, including Flemming's weaker solution, chromacetic acid, and picrosulphuric acid, were tried, but the best results followed from a saturated solution of mercuric chloride in 70 per cent. alcohol, used hot. This caused no collapse, and seemed to penetrate the thick transparent mucilage envelope round the thallus very quickly. Sections 2-6  $\mu$  thick were cut after embedding in paraffin wax, but the most striking nuclear figures were found in whole material mounted in venetian turpentine, which rendered the cells very transparent. Heidenhain's iron haematoxylin, without counter-stain, was used throughout, though other stains were also tried. Material was fixed at different times of day and night.

*Cell Organisation.*

*D. glomerata* is characterized by the differentiation of the thallus into stem-like axes with little chlorophyll, and bright green lateral branches consisting of tufts of assimilating filaments ending in colourless hairs. The cells of the main axis are  $45\text{--}60\ \mu$  long and about  $40\ \mu$  wide; each cell contains a single nucleus, several pyrenoids, and an equatorial belt-like chloroplast. The nucleus usually lies close against the cell-wall in the peripheral protoplasm. The cells of the branchlets are much smaller, about  $10\ \mu$  long and  $5\ \mu$  wide. The chloroplasts are relatively much larger and the pyrenoids are more prominent. Spore formation is restricted to these cells.

The nuclei are about  $2\ \mu$  in diameter, and in the branchlet cells are not easily recognized. They occupy a central position and are suspended in the cell by strands of cytoplasm which stain rather deeply. Often the presence of this dark bridle round the nucleus is the best means of distinguishing it from the pyrenoids, for the nucleolus often stains in exactly the same way as the central body of the pyrenoid. More than sixty cases of division have been observed, and though many have been found in material fixed in the late afternoon (4.30–6 p.m.) mitotic figures occur most abundantly in batches fixed about 10.30 a.m. All the cells of a filament do not divide simultaneously. In a branch consisting of six cells the lowest may contain a resting nucleus, the next above it a dividing nucleus, and the four upper cells may show signs of recent division (Pl. XXVI, Fig. 1). Thus nuclei in very different phases may be found scattered through the material. The transverse septa of the filaments are often covered with deeply-staining granules.

*The Resting Nucleus and Prophase.*

The resting nucleus takes a light stain and contrasts with the dark surrounding cytoplasm. The nucleolus is distinct though not deeply stained, and there are faint indications of a fine reticulate structure in the body of the nucleus (Pl. XXVI, Fig. 2). The first indication that division is about to take place is a marked increase in the size of the nucleus, which at this stage is very lightly stained (Pl. XXVI, Fig. 5). Next tiny granules appear all over the surface of the nucleus and the nucleolus disappears. At this and at later stages in the process the pyrenoids are inconspicuous, and in most cases almost unstained. Soon the granules gather together and become grouped in masses which foreshadow the chromosomes to be formed from them. At this stage (Pl. XXVI, Fig. 4) the nucleolus has disappeared, and the granules take the deep stain of chromatin structures. The chromosomes, eight in number, are formed by condensation of the granules without the intervention of any spireme stage.

This is in agreement with certain other records of nuclear division in the green algae. The chromosomes are small rounded bodies or very short rods, which show up clearly in the light unstained area in the cytoplasm originally occupied by the resting nucleus. They show signs of fission at an early stage. Pl. XXVI, Fig. 5, shows a case in which four of the eight chromosomes have split across before metaphase, while they are still moving irregularly towards the equatorial region.

#### *Metaphase.*

At this stage the chromosomes are arranged in one plane and are easily counted. All the chromosomes have split, so that sixteen bodies lying in pairs can be seen (Pl. XXVI, Figs. 6, 7, 8). In Fig. 7 the chromosomes appear slightly longer than in most other cases observed, and two of the daughter chromosomes are noticeably rod-shaped. Only two cases of division in the nuclei of axis-cells have been found. One appears to be in early prophase condition and shows a ring of deeply stained material in the nucleolus (Pl. XXVI, Fig. 3); the other is at metaphase (Pl. XXVI, Fig. 9). Here the nucleus is rather larger, and the chromosomes are longer and more varied in shape than in the branch-cells. There are eight chromosomes; one long and somewhat S-shaped, four rather long rods distinctly constricted across the middle, and two short transversely constricted rods, one of which lies over one of the other chromosomes. The other cells of the axis contain nuclei with prominent nucleoli.

#### *Anaphase.*

Before the separation of their halves the chromosomes are orientated parallel to the longer axis of the cell. An early stage is shown in Fig. 10, where there are two rings of daughter chromosomes, eight in each ring, viewed obliquely so that they appear to overlap. The daughter chromosomes, which are always almost spherical in shape, then move apart towards the poles. Sometimes they move very evenly (Fig. 11). As the daughter chromosomes are usually well spread out they are quite easily counted (Pl. XXVI, Figs. 12, 13, 14). Pl. XXVI, Fig. 15 is taken from a section and may be interpreted as a view of one of the polar groups.

#### *Telophase.*

This phase is found more often than any other. The chromosomes clump together and gradually lose their individuality, and sometimes one lags behind the rest in its passage to the pole and remains distinct a little longer (Pl. XXVI, Figs. 13, 16). The nuclear membrane begins to reappear when the chromatin is still in irregular masses, and is fully formed when the nucleolus is reconstituted (Pl. XXVI, Fig. 17). A recently formed nucleus

has a large nucleolus which takes an intense stain, but later the body of the nucleus becomes somewhat granular, the nucleolus loses its marked affinity for haematoxylin, and the resting condition is reached (Pl. XXVI, Fig. 18). The new wall formed is not laid down during nuclear division, but extends inwards as a collar from the original cell-wall. Ultimately it separates the newly organized daughter nuclei which at first lie close together in the middle of the cell.

#### *Pyrenoids.*

The pyrenoids vary in number from two to five in each branch-cell, and in stained material they differ considerably in appearance from time to time. Usually the effect produced is of a solid black mass, often angular in form, surrounded by a white unstained sheath. At other times the central body appears to be broken up into a number of irregular fragments still surrounded by this sheath, or the central body may be represented by a network of stained material with darker granules at the corners of the mesh. Recognizable pyrenoids may disappear, but the cell may be full of minute deeply stained granules.

A correlation between the staining reactions of the pyrenoid and the condition of the nucleus has been noticed. When the nuclei are in resting condition and barely take up a haematoxylin stain the pyrenoids are often deeply stained and are heterogeneous, with prominent unstained sheaths. When the nuclei are in active division the pyrenoids are either small or very faintly stained. In one batch of material practically all the nuclei underwent division simultaneously, for some reason not understood. Many pairs of nuclei, obviously products of recent division, were seen throughout the material, but no pyrenoids could be found. This was the more striking because the abnormal appearance of the filaments was noticed when they were in the fresh condition, when the pyrenoids are normally very conspicuous, and subsequent fixation and staining confirmed the absence of the pyrenoids. In certain algae, where each cell contains a single pyrenoid, the central body divides and a portion passes into each daughter-cell formed after the division of the nucleus. In *Draparnaldia*, where there are several pyrenoids in each cell, no definite cycle such as this seems to occur, and though extensive fragmentation takes place this does not seem to be connected with any special stage in cell formation. Zoospores contain several pyrenoids when released.

#### DISCUSSION.

An interesting point in this account of nuclear division in *Draparnaldia* is the absence of any definite spindle. In only one case (Fig. 14) has there been the slightest indication of spindle structure. In all other cases the chromosomes move in the clear region in the middle of the cell, and this



agrees with the report of T'Seracles (7) for *Cladophora glomerata* and that of Higgins (2) for *C. flavescens*. Carter (1) found only a few doubtful spindles in her work on the Ulvaceae, but believes that they may sometimes be present. In some respects, i.e. formation of chromosomes from granules without any spireme stage, the shape of the chromosomes, and the absence of spindle, there is agreement with von Neuenstein's account of mitosis in *Microspora amoena* (5).

As yet it is impossible to decide whether the chromosome number, i.e. eight, which has been invariably found, represents the haploid or the diploid condition. In most accounts of *Draparnaldia* it is stated that the branch-cells produce quadriciliate spores, one to four from each cell, and that these may fuse in pairs before germinating, or may develop into new filaments without any fusion.<sup>1</sup> From this, and in view of Kniep's statement that microzoospores developing parthenogenetically give rise to two plants while zygotes produce four, it might be supposed that the complete life-cycle involves meiosis at some stage, either before gamete formation, or, more probably, in the early divisions of the sporeling. Theoretically there may exist three types of sporeling, developed (*a*) from a zoospore (Klebs's macrozoospore), or (*b*) from a zygote produced as a result of fusion, or (*c*) parthenogenetically from an unpaired gamete. Types (*b*) and (*c*) would presumably be diploid and haploid respectively unless meiosis follows immediately after zygosis, when both would be haploid; type (*a*) would resemble its parent thallus in chromosome constitution.

As yet, however, there is no positive evidence on this point. Although the liberation of countless spores has been watched, there have been no cases of fusion, and no cell has produced more than one spore. Variation in size among the spores exists, but this is due only to differences in size between the parent cells, and the smaller spores do not behave as gametes as did the microzoospores described by Klebs (3). This absence of fusion between spores, a process very frequently described, is somewhat puzzling. It may be that for certain conditions of habitat or nutrition a purely asexual habit has become established, and that neither fusion nor reduction takes place. Such a cycle might arise in either phase, and until nuclei giving some other number of chromosomes are found, no further conclusion can be reached.

#### SUMMARY.

1. Mitotic division in *D. glomerata* is described and the chromosome number is found to be eight. Neither spireme nor spindle has been seen, and the very small chromosomes result from the coalescence of still smaller granules.

<sup>1</sup> Klebs (3), Pascher (6), Kniep (4), West and Fritsch (8).

2. A relationship between the staining reactions of pyrenoid and nucleus is noted.

3. The escape of quadriciliate spores of different sizes has been watched on many occasions, but there is no evidence that the smaller spores behave as gametes. No fusions have been recorded and the branch-cells have not produced more than one spore each. The bearing of these observations on the possible life-cycle of the alga is discussed.

The writer wishes to express her thanks to Professor B. H. Bentley and Dr. E. M. Lind for their valuable advice and criticism throughout the progress of this work, which was carried out in the Botany Department of the University of Sheffield during the tenure of a maintenance grant from the Department of Scientific and Industrial Research.

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#### EXPLANATION OF PLATE XXVI.

Illustrating Dr. Ferguson's paper On the Mitotic Division of *Draparnaldia glomerata*.

All figures drawn with the aid of a Zeiss camera lucida. Most of these stages are from whole material mounts in venetian turpentine.

Fig. 1. Diagram showing progress of division in filament.

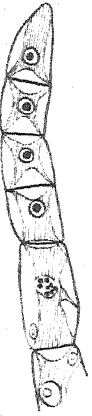
Fig. 2. Resting nucleus, showing faint reticulate structure, cytoplasmic bridle and pyrenoid.  $\times 1,200$ .

Fig. 3. Nucleus from axis cell. Ring of chromatin in nucleolus.  $\times 996$ .

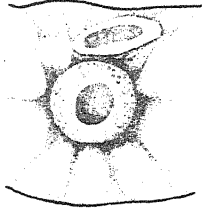
Fig. 4. Late prophase. The nucleolus has disappeared, and groups of chromatin granules have developed.  $\times 1,200$ .

Fig. 5. Chromosomes formed and already showing splitting. Pyrenoids and the nucleus of the adjacent cell very little stained.  $\times 1,080$ .

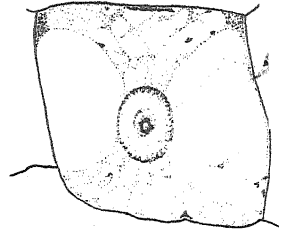
- Fig. 6. Metaphase plate, polar view.  $\times 2,250$ .  
Fig. 7. Another metaphase plate.  $\times 1,080$ .  
Fig. 8. Metaphase, all eight chromosomes split. Pyrenoids very faint, nuclei in two upper cells just returned to very slightly stained 'ghost' condition after division.  $\times 1,500$ .  
Fig. 9. Metaphase in axis cell.  $\times 2,250$ .  
Fig. 10. Early anaphase, polar view. Two rings of chromosomes beginning to separate.  $\times 2,250$ .  
Fig. 11. Anaphase.  $\times 2,250$ .  
Fig. 12. A fairly late stage in anaphase, but the chromosomes are well separated.  $\times 1,494$ .  
Fig. 12a. Later stage with groups of chromosomes approaching poles.  $\times 2,250$ .  
Fig. 13. Anaphase.  $\times 1,000$ .  
Fig. 14. Early telophase, chromosomes beginning to clump together, faint signs of spindle.  $\times 1,000$ .  
Fig. 15. One group of chromosomes at the pole, from a section.  $\times 2,250$ .  
Fig. 16. Telophase, chromosomes less distinct than in Fig. 14.  $\times 1,500$ .  
Fig. 17. Late telophase, complete fusion of chromosomes.  $\times 1,500$ .  
Fig. 18. Daughter nuclei returning to resting condition. Wall beginning to extend between them.  $\times 600$ .



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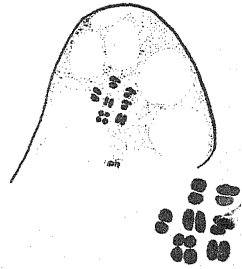
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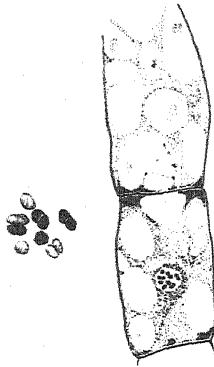
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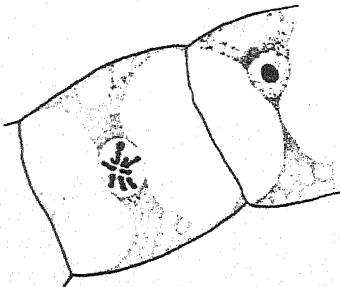
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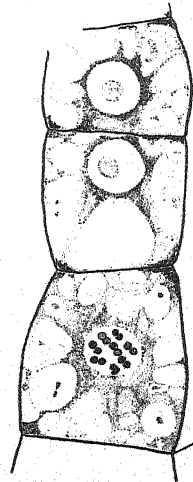
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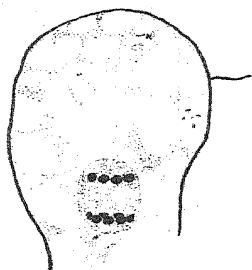
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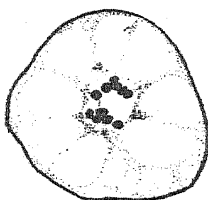
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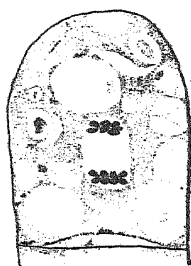
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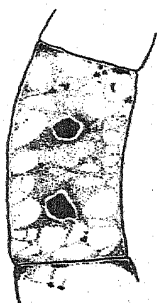
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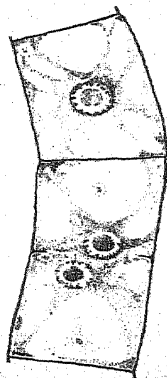
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18



# A Contribution to the Life-history and Cytology of Two Species of *Ulothrix*.

BY

EDNA M. LIND, PH.D.

(*Department of Botany, University of Sheffield.*)

With Plates XXVII and XXVIII and twelve Figures in the Text.

## INTRODUCTION.

OUR knowledge of the genus *Ulothrix* has hitherto been almost entirely confined to the one species *U. zonata*. The investigations of Dodel (2), Klebs (8), Pascher (16), and others on this alga have revealed the fact that *U. zonata* reproduces by macrospores which are asexual and by gametes which fuse in pairs, or may develop parthenogenetically.

Macrospores may be formed in any cell of a filament, are quadriciliate, and on germination give rise directly to new filaments. Gametes are also produced by any cell of a filament, but in much larger numbers. They are biciliate, fuse in pairs, and the resulting zygotes after a period of rest give rise to spores from which new filaments develop. If gametes fail to fuse they may form resting spores and germinate later. Klebs and Pascher describe a third type of spore, a microspore, which stands morphologically midway between macrospore and gamete, has two or four cilia, and is never seen to fuse.

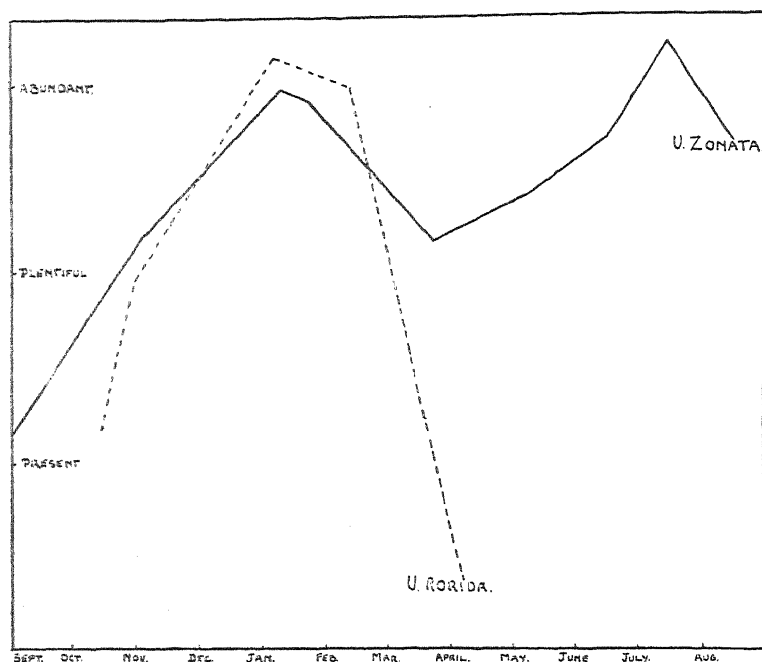
There seems therefore to be a gradual transition both morphologically and physiologically from the definitely sexual to the definitely asexual form of spore. As all three types of spore arise from divisions of the ordinary vegetative cell it seemed likely that an investigation of the cytology of the spore-forming filaments might throw more light on the life-cycle of the genus *Ulothrix*.

## *Description of Species.*

*U. zonata* (Kütz) was found growing on a slab beside a stone water-trough in the village of Dore in Derbyshire. A close examination of this material at different times of the year revealed the presence of two distinct

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species of *Ulothrix* on the slab. One consisted of filaments 10 to 45  $\mu$  in diameter with several pyrenoids per cell and fairly thick walls, especially in the older filaments. This agrees in every way with the descriptions of



TEXT-FIG. 1. Diagram showing relative abundance of *U. zonata* and *U. rorida* throughout the year.

*U. zonata*. The filaments of the other species had an average diameter of about 14  $\mu$  and never exceeded 20  $\mu$ . They had thin walls and never more than one pyrenoid per cell (Pl. XXVIII, Fig. 18). This species does not agree closely with any of the forms described in the systematic works on algae. It comes near in size and form of cell to *U. aequalis* (Kützinger), but differs in the structure of its chloroplast, which forms a complete band round the cell, and in the possession of only one pyrenoid. The latter feature is most constant.

I believe this is probably the same species as was found by Gross growing mixed with *U. zonata* and figured in her paper (14). She considers it to resemble *U. oscillarina* (Kützinger), except that this again has two pyrenoids.

Thuret (22) describes a species which he names *U. rorida* and which seems to agree most closely with the form quite common in several places near Sheffield. His figures show that it is easily distinguished from *U. zonata* both by its spindle-shaped spores and by the form of its vegetative cells. Still another description of the same species occurs in Klebs's paper (8),



but he considers it to be a form of *U. zonata* occurring mainly in spring, and distinguished by its more delicate filaments and strongly coiled gamete-forming filaments. Its spindle-shaped spores he believes to represent the zygotes of *U. zonata*.

It is of interest that this species, to which it is proposed to give the name *U. rorida* suggested by Thuret, has been recorded now from three different countries, and that in each case it was found growing mixed with *U. zonata*. It seems possible that some of the variation in size and structure and in spore form attributed to *U. zonata* may be due in part to confusion which has arisen between these two species.

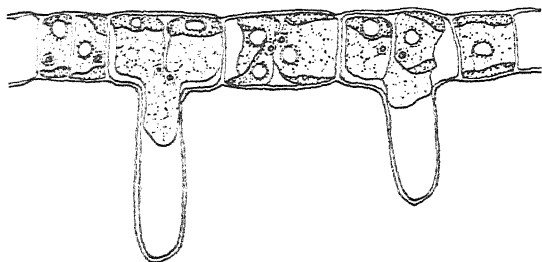
A careful search of the streams in the neighbourhood revealed three more habitats for *U. rorida*, and I have not the slightest doubt that this is a definite and well-defined species. It is easily distinguished from the larger filaments of *U. zonata* but the resemblance between the young filaments of the two species is very strong. *U. zonata*, however, is always marked by the presence of more than one pyrenoid in some of its cells and by the restriction of the chloroplast to the more central part of the cell-wall. Moreover it is frequently coated with bacteria, diatoms, and other epiphytic organisms, and sometimes with small sporelings, a feature only rarely observed in *U. rorida*. As is shown in the diagram (Text-fig. 1) the two species reach a maximum at different times of the year.

#### *Ulothrix zonata*.

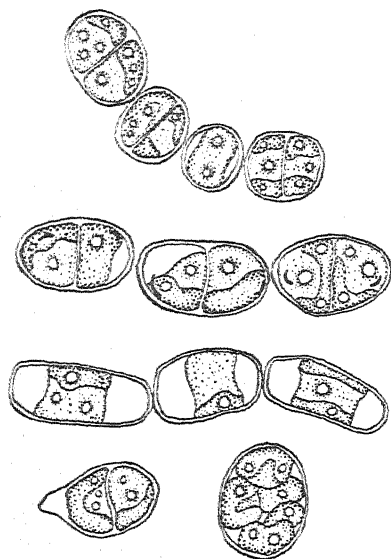
In an account of the life-history of *U. zonata* recently published by Gross (4), the cytology of the spore-forming cells is described and reduction division is shown to take place in the germination of the zygote. I have as yet little to add to her observations, except with reference to the occurrence of microspores and to abnormalities in the growth of the alga which took place in culture and which I have observed under natural conditions.

Reproduction is normally by spores, which on germination give rise directly to filaments attached by a single terminal rhizoid. Very frequently filaments were found in which secondary rhizoid-like outgrowths had developed at intervals, sometimes as many as fourteen on one filament (Text-fig. 2). These were most noticeable in the older broad filaments. Gross found the same effect when the alga was grown for too long on the same medium. It seems reasonable to suggest that these secondary outgrowths represent an increase of absorbing surface under conditions where nutriment is deficient. Branching of the filaments occurred in sporelings growing on a glass slide in a tube of standing water (Pl. XXVIII, Fig. 23). Some of the broader filaments were attached only by a holdfast developed in the middle of the filament. I believe that these may be the products of vegetative reproduction.

Some material collected in May was examined after standing for a week in a tube in the window. The filaments were still healthy and green, but had begun to fragment into pieces consisting of one cell, two newly

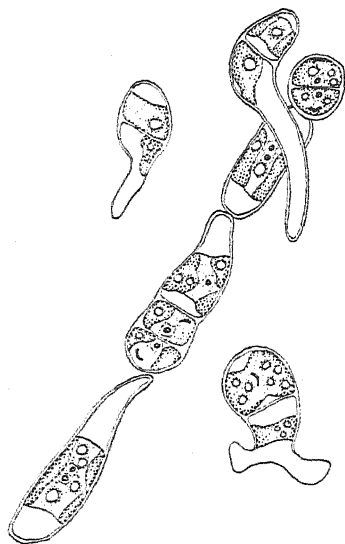


TEXT-FIG. 2. Two out of fourteen secondary rhizoids on one filament of *U. zonata*.  
× 350.



TEXT-FIG. 3.

TEXT-FIG. 3. *U. zonata*. Fragmentation of filaments. × 300.

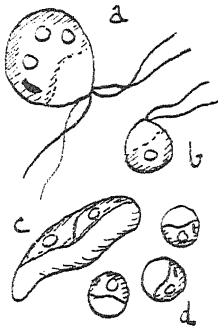


TEXT-FIG. 4.

TEXT-FIG. 4. *U. zonata*. Development of rhizoid-like outgrowths from the fragments.  
× 300.

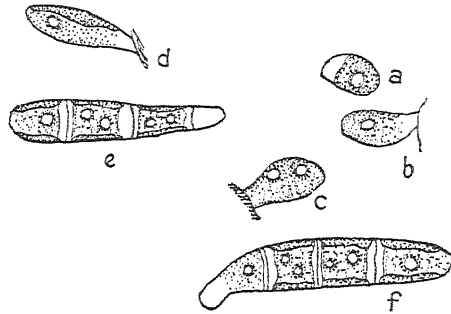
formed cells, or cells containing spores (Text-fig. 3). In each case the cell-wall was considerably thickened. The same material a day or two later showed the fragments elongating to form at one end colourless tube-like outgrowths, which in a few cases were branched (Text-fig. 4). A month later numerous short filaments were formed in the tube. The chloroplasts were contracted but still bright green, and the filaments possessed thick walls with a sheath discontinuous in many places (Pl. XXVIII,

Fig. 22), and they showed a tendency to further fragmentation. I have not seen this fragmentation occur in nature and believe it to be a response to unfavourable conditions. A similar condition of the alga is described by



TEXT-FIG. 5.

TEXT-FIG. 5. *U. zonata*. a. Macrospore. b. Microspore. c. Resting macrospore. d. Resting microspore.  $\times 600$ .



TEXT-FIG. 6.

TEXT-FIG. 6. *U. zonata*. Germinating spores after one week. a, b, c, d. Microsporelings. f, e. Macrosporelings.  $\times 600$ .

Chodat (1), while Gross observed fragmentation to take place in culture when the temperature rose to  $14^{\circ}$  or  $15^{\circ}$  C. I am not yet able to say what happens to these peculiar thickened fragments when conditions become normal again.

*Spore formation.* On the origin, structure, and development of macrospores I have little to add to existing descriptions. There would seem, however, to be some doubt as to the existence of the microspores described by Klebs (8). Gross states that she failed to observe microspores, but that all biciliate spores fused in pairs before developing further. These biciliate gametes were of two strains, and those from the same filament never fused. Pascher (16) describes microspores, the larger among which resembled macrospores. They were mainly quadriciliate, and only 15–25 per cent. of those below  $8\mu$  in diameter were biciliate. In February of this year, in the same large filament which was producing macrospores there appeared much smaller spores in swollen and rounded cells and considerably more than sixteen per cell (Pl. XXVIII, Fig. 19). When released the spores remained together in groups of two, four, or more, and then broke free and moved swiftly away from the filament. They were biciliate but never fused (Text-fig. 5). Macrospores and microspores were caught in large numbers on slides and left to germinate under natural conditions in a stream. The microspores germinated much more slowly than the macrospores. After a week, when the macrosporelings were many-celled, the microsporelings showed only an elongated body of never more than two cells (Text-fig. 6; Pl. XXVIII, Fig. 20). Gradually, however, they

developed into narrow filaments with the chloroplast covering nearly the whole cell-wall and often with only one pyrenoid per cell (Pl. XXVIII, Fig. 21). After a few weeks they produced small quadriciliate spores, eight per cell. There were very many filaments releasing microspores at the same time, so I do not think their failure to fuse can have been due to the lack of two complementary strains. In everything but their shape, which he describes as spindle-shaped, and in the constant possession of two cilia they resembled the microspores of Klebs. Like the macrospores they frequently germinated within the cells of the filament.

*Ulothrix rorida.*

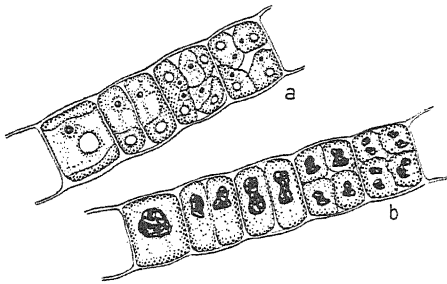
The alga is found in quantity and unmixed with any other species in Graves Park, Sheffield, on the stone weir over which the overflow water from the big lake falls into the stream below. The dense, bright green mats of filaments are attached firmly to the rock, mainly in the grooves, and on that side of the groove which is opposed to the direction of flow of the water. The alga is never more than 1 in. below the surface and has therefore good aeration and illumination.

The adult filaments are from 13 to 18  $\mu$  in diameter and anything up to 5 in. in length. They are made up of cells which are about as long as broad, though where cell-division is taking place there are always to be found elongated cells which are about to divide, and newly formed short daughter-cells (Pl. XXVIII, Fig. 18). The sporelings, though narrower, are similar in appearance. The filaments are attached by colourless terminal holdfasts, and only occasionally are secondary holdfasts found at other parts of the filaments.

Each cylindrical cell is bounded by a thin cellulose wall. In the protoplasmic lining is embedded a single continuous belt-like chloroplast apparently coincident with the cytoplasm. The nucleus is placed slightly to one side and there is a single large pyrenoid in each cell.

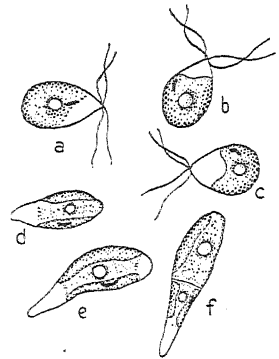
*Spore formation.* Previous to spore formation the cell contents contract slightly and become full of reserve material. The nucleus divides and the pyrenoid, which has become very large, fragments laterally into two halves. The division of the nucleus and pyrenoid is followed by the cleavage of the cytoplasm and chloroplast into two halves in a plane at right angles to the long axis of the filament (Text-fig. 7). The two new pyrenoids now become much elongated transversely until each occupies about three-quarters of the width of the cell, when each again fragments. The two nuclei divide again, and a further cleavage of the cytoplasm gives four spores, each with a nucleus and a single pyrenoid. Nucleus and pyrenoid may divide twice before cleavage occurs. The third division of the cytoplasm appears to be parallel to the long axis, but in a plane at right angles to the second cleavage, so that the eight spores are now

arranged in two layers. The pyrenoids multiply each time by fragmentation. The eye-spot does not make its appearance till after the final cleavage in the cell. The zoospores do not arise simultaneously as Strassburger states (21).



TEXT-FIG. 7.

TEXT-FIG. 7. *U. rerida*. Division of (a) Nucleus, (b) Pyrenoid, during spore-formation.  $\times 550$ .



TEXT-FIG. 8.

TEXT-FIG. 8. *U. rerida*. a, b, c, zoospores. d, e, f, zoospores germinating.  $\times 600$ .

*Macrospores.* Macrospores are produced usually 8 or 4, or, rarely, 2 per cell. They do not always arise in even numbers or in multiples of two, presumably because the divisions are not completed before the spores escape. The spores vary in size from  $7.5$  to  $13\ \mu$  long by  $5$  to  $6.5\ \mu$  wide and are always quadriciliate (Text-fig. 8). The chloroplast covers more than three-quarters of the wall and contains a single large pyrenoid in the lower half. The larger spores are spindle-shaped and only slightly pointed at the posterior end, while the smaller ones are more rounded. The conspicuous eye-spot is situated nearer the anterior end. When the spores are ripe, the wall of the cell bulges and then breaks down, and the spores escape, surrounded by a vesicle which soon bursts. After swimming about rapidly they settle down to oval bodies lying on their sides and germinate at once to give new filaments.

*Gametes.* The gamete-forming filaments are strongly coiled and therefore easily distinguished (Pl. XXVIII, Figs. 16 and 17). The formation of gametes begins towards the apex of the filament and proceeds towards the base till nearly every cell is swollen, slightly barrel-shaped, and packed with gametes. The preliminary divisions of the cell are exactly as in the spore-forming cells, except that the divisions are carried further, resulting in the formation of 16 or more gametes.

The gametes escape, surrounded by a vesicle within which they move for about 30 seconds before becoming free. They do not swim far from the filament, but perform jerky movements within a limited area and

quickly fuse in pairs. Gametes from the same cell were never seen to fuse, but gametes from neighbouring cells of the same filament fused freely. The gametes are biciliate, rounded or slightly spindle-shaped, and  $5\mu$  in diameter. The chloroplast is situated in the lower half rather to one side and the eye-spot is about half-way down (Text-fig. 9 a).

After swimming about for a few seconds the gametes approach end to end and become entangled by their cilia. The double mass moves rapidly about, spinning on its long axis (Text-fig. 9 b), until gradually one spore comes to lie alongside the other (Text-fig. 9 d), the wall between the two disappears, and an almost spherical body with four cilia results (Text-fig. 9 f). It is clear and colourless except at one end, where two distinct chloroplasts with their eye-spots are visible. The zygote at first rotates on its own axis, then moves about clumsily and settles down. The mortality rate among zygotes formed under the unfavourable conditions of a laboratory is very high. A number of glass slides were left in the stream at the period of gamete formation, and in zygotes caught on these slides the early stages of germination were observed. By the fifth day the rounded zygote had put out a faintly coloured rhizoid-like structure and contained one pyrenoid and two distinct eye-spots, one in the green body and one in the rhizoid (Text-fig. 9 g, h). After this the zygotes died, and I have not been able to follow their development further.

*Cytology.* Until recently it was considered that the individual plants of all Chlorophyceae were haploid. During the last five years it has been shown that in the case of *Cladophora* (17, 6), *Chaetomorpha* (5), *Ulva* (3), and *Enteromorpha* (5), there is an alternation of generations, reduction division taking place during the formation of zoospores. These spores give rise directly to haploid plants which show sexual differentiation. Gametes produced from plants of different sex fuse in pairs, and from the zygotes the new diploid plants arise. In *Codium* (24), *Acetabularia* and *Cladophora glomerata* (18), the reduction division occurs in the gamete-forming cells. An investigation of the cytology of *Ulothrix rorida* was undertaken in order to discover the nuclear phases in the life-cycle.

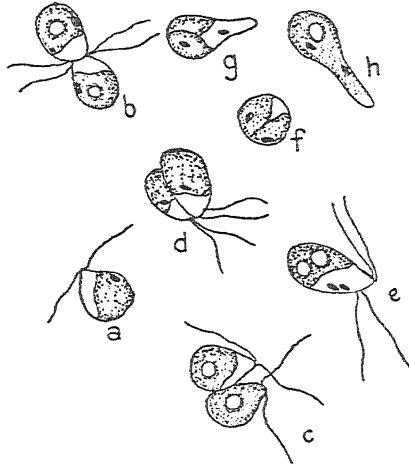
*Division of the vegetative cell.* Material was fixed at half-hourly intervals from 7 a.m. to midnight in a chrom-acetic mixture of the following proportions:

Chromic acid . . . .	1 gm.
Glacial acetic acid. . . .	3 c.c.
Osmic acid, 1 per cent. . . .	6 c.c.
Water . . . . .	100 c.c.

Vegetative divisions were most frequent at 10 p.m., becoming fewer up to midnight, and very scarce during the daytime.

The resting nucleus has a clearly defined nucleolus and a much less

clearly defined peripheral region in which only a very faint network is discernable. Previous to division the nucleus enlarges greatly, and an early prophase (Pl. XXVII, Fig. 1) shows the nucleolus laterally placed, sur-

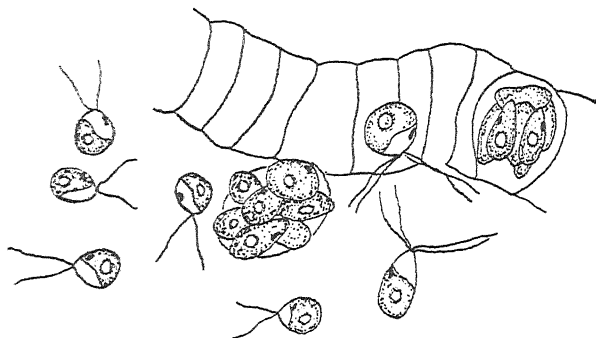


TEXT-FIG. 9. *U. verridis*. a. Gamete. b, c, d, e. Stages in fusion of gametes. f. Zygote. g, h. Zygotes germinating.  $\times 600$ .

rounded by a fine reticulum of scattered chromatin dots. The dots become fewer and larger (Pl. XXVII, Fig. 2) and arrange themselves near the equator (Pl. XXVII, Fig. 3). It is at this stage that the chromatin material coalesces to form five chromosomes, which immediately divide so that the metaphase shows five already split chromosomes arranged on a plate (Pl. XXVII, Fig. 4). A spindle shows clearly at this point, and the five daughter chromosomes pass to the poles (Pl. XXVII, Figs. 5, 6), where they form into two groups (Pl. XXVII, Fig. 7) and gradually lose their identity in the reconstitution of two daughter nuclei. The first cleavage of the cytoplasm appears at the late anaphase (Pl. XXVII, Fig. 6) and passes gradually inwards from the periphery. The pyrenoid is very large, and stains deeply at the time when division begins. During the stages of division it is very faint and becomes elongated parallel to the long axis of the cell, but the actual division of the pyrenoid does not occur until the daughter nuclei are almost fully formed.

*Nuclear divisions during gamete and spore formation.* Gametes are formed from any cell of a filament. At the height of gamete formation practically every cell of the older filaments except the rhizoid shows either ripe gametes or early stages in their development. Previous to the production of gametes the cell contents become slightly contracted and stain rather more deeply than the vegetative cells (Pl. XXVII, Fig. 8). Stages in the first division of the nucleus show that this is a simple mitosis similar to that of the vegetative cell (Pl. XXVII, Fig. 5, 8, 9, 11). Later divisions in

the gamete-forming cells still show the five chromosomes in ana- and telophase (Pl. XXVII, Figs. 10, 12). The division of the nucleus in the spore-forming cells is hardly distinguishable from that just described for the cells producing gametes (Pl. XXVII, Figs. 13, 14, 15).



TEXT-FIG 10. *U. vorida*. Quadriciliate and biciliate spores arising from the same filament.  $\times 600$ .

There seems no doubt that in *U. vorida* all the plants are haploid and that both gametes and zoospores have the haploid number of chromosomes. As gametes fuse in large numbers to give zygotes it seems probable that the zygote represents the only diploid phase in the life-history. Unfortunately the nuclear divisions during its germination have not yet been observed.

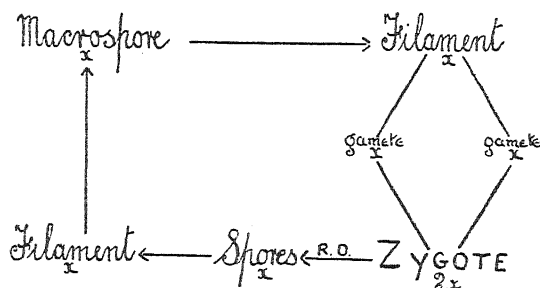
*Relationship between spores and gametes.* A study of the relationship between spores and gametes and of the periodicity of their formation confirms the opinion that all types of spore are formed from haploid filaments. Morphologically there is no clear dividing line between spores and gametes. From filaments producing true gametes at least 16 per cell were also released spores similar in appearance and origin to gametes but arising only 8 per cell. They were undoubtedly biciliate, but were very rarely seen to fuse. They had not the same individuality as the microspores of *U. zonata* described above and were not produced from macrospore-forming filaments. They are to be looked upon as potential gametes which have been released (possibly owing to the advent of unnatural conditions) before they are mature.

From another gamete-forming filament were further released a number of quadriciliate spores (Text-fig. 10). Some of them arose 16 per cell and were somewhat rounded, while others arose 2, 4, or 8 per cell and were spindle-shaped and very active. If all the filaments are haploid, then both gametes and macrospores have the haploid number of chromosomes. This is in accordance with the observations already described. Further, if gametes and spores are to be produced from the same filament, *either* the cells of that filament and all the spores produced are haploid, *or* the cells



of the filament are diploid and reduction division takes place in the formation of gametes. No reduction has been observed in any of the spore- or gamete-forming cells.

Until the germination of the zygote has been studied these observations on the life-history of *U. rorida* will be incomplete. It seems, however,



TEXT-FIG. 11.

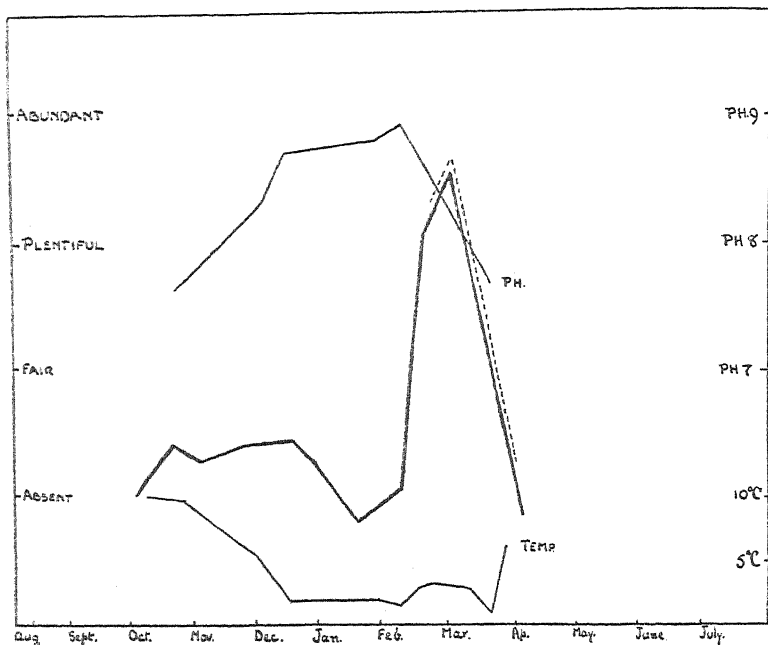
very probable that the zygote represents the only diploid phase and that the life-cycle can be summed up in the accompanying diagram (Text-fig. 11). Gross (4) finds this to represent also the life-cycle of *U. zonata* except that in this species the gametes are of two strains.

*Periodicity of spore-formation.* Material of *U. rorida* was first collected in March 1928 when it was liberating gametes. Gamete formation increased up to the end of March and then fell off. Regular collections were made throughout the autumn and winter of 1928-29-30-31. Each year spores were found whenever the material was in good condition, but no gametes ever appeared until the middle of February. Further, the change from spore to gamete formation took place quite suddenly.

On February 11 not a single gamete was seen, though spores were plentiful. A week later the strongly coiled gamete-forming filaments were so numerous as to be visible to the naked eye. Each year the change took place within a week, and on each occasion there was a frost and bright sunshine during that period. In the spring of 1931, when the sudden transition was most marked, the lake was frozen and the temperature of the water flowing over the weir was never much above freezing-point. Six inches of snow fell, and there was bright sunshine every day. It seems probable that filaments which are engaged in forming zoospores change suddenly to gamete production by further subdivision of some of their cells. The same rapid change to gamete formation during bright sunshine has been described for *U. zonata* (14).

As a result of these observations one is inclined to agree with Dodel's suggestion for *U. zonata* that the formation of macro- or microspores does not depend so much on the nature of the filament as upon external circumstances. In many of the lower plants the production of sexual

organs coincides with the arrival of conditions unfavourable to growth. Yet low temperature and bright sunshine would not appear to be adverse factors. Indeed it is suggested that the further subdivisions of the cell-



TEXT-FIG. 12. *U. vorrida*. Diagram showing relative abundance of the alga throughout the year, fluctuations of pH and of temperature. The dotted line indicates the period during which gametes were produced.

contents necessary for gamete formation may be rendered possible by the increased photosynthetic activity of the cells under good illumination. But each year the production of gametes marked the close of the life-period of the alga. Observations on the hydrogen ion concentration of the water have only been carried out during one season, when it was noticeable that the beginning of gamete formation coincided with the fall in the hydrogen ion concentration of the water (Text-fig. 12).

*Summary and conclusion.* The observations on the life-history of *U. vorrida* and of *U. sonata* described above lead to the following conclusions:

*U. vorrida* is a well-defined species and not a form of *U. sonata*. All the filaments are haploid, producing in autumn and winter quadriciliate asexual zoospores, and in the early spring biciliate gametes. Both spores and gametes have the haploid number of chromosomes and may be formed in adjacent cells of the same filament. There is no differentiation between the gametes, so that gametes from the same filament fuse freely in pairs. The zygote represents the only diploid phase in the life-cycle, and reduction division probably takes place at its germination.

SUMMARY.

1. Two species of *Ulothrix* found growing together in one locality are described. It is suggested that one is *U. zonata* (Kütz) and the other a form named *U. rorida* by Thuret.

2. The reaction of *U. zonata* to unfavourable conditions of environment is described, together with a form of vegetative reproduction by fragmentation of the filaments.

3. An account is given of observations on the structure, occurrence, and germination of the microspores of *U. zonata*.

4. A description of *U. rorida* is given, together with an account of its life-history and the cytology of its reproductive cells. It is shown that all the filaments and the zoospores and gametes which they produce are haploid, and that the zygote represents the only diploid phase in the life-cycle.

APPENDIX.

*The Development of the Zygotes of Ulothrix rorida.*

Since the completion of this paper, the zygotes of *Ulothrix rorida* have again been under observation. A number of zygotes were caught on coverslips, kept in a stream of aerated water and fixed at intervals for a fortnight. After one day a number of zygotes still showed two distinct nuclei indicating that the fusion of the nuclei of the gametes may be somewhat delayed. After three days the zygotes all showed two pyrenoids and one nucleus, the same condition being found at the end of a fortnight. The small tube-like outgrowth described and figured on pp. 718 and 719 was observed only in a few cases.

The remaining coverslips with their zygotes were kept in a stream of aerated water under artificial illumination in the greenhouse for two months, and when the temperature began to rise they were transferred to a stream in a tube closed at both ends with bolting silk. After five weeks in the stream the whole tube was washed away during very heavy rain.

When last examined the rounded zygotes had increased slightly in size and were uniformly green, the division between the chloroplasts being no longer visible.

The duration of the resting stage of the zygote could not be ascertained but there was no evidence of reduction division taking place shortly after the formation of the zygote as would appear to be the case in *Ulothrix zonata* (4).

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## EXPLANATION OF PLATES XXVII AND XXVIII.

Illustrating Dr. E. M. Lind's paper on 'A Contribution to the Life-history and Cytology of Two Species of *Ulothrix*'.

## PLATE XXVII.

Nuclear division in the cells of *Ulothrix virida*.

Figs. 1-7 Vegetative cell-division.

Fig. 1. Early prophase.  $\times 1,875$ .

Fig. 2. Late prophase.  $\times 1,875$ .

Fig. 3. Chromatin material collecting previous to metaphase.  $\times 1,875$ .

Fig. 4. Metaphase. Chromosomes just splitting.  $\times 1,875$ .

Fig. 5. Early anaphase.  $\times 1,875$ .

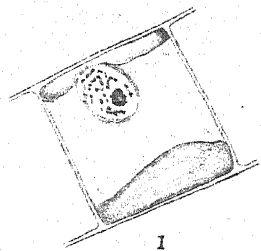
Fig. 6. Late anaphase. Beginning of cleavage of cytoplasm.  $\times 1,875$ .

Fig. 7. Telophase.  $\times 1,875$ .

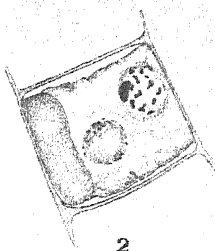
Figs. 8-12. Division of nucleus of gamete-forming cell.

Fig. 8. Prophase.  $\times 1,750$ .

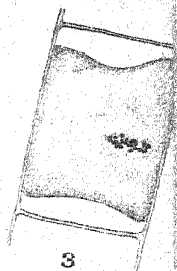
Fig. 9. Late metaphase.  $\times 1,750$ .



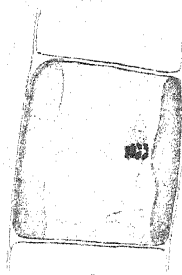
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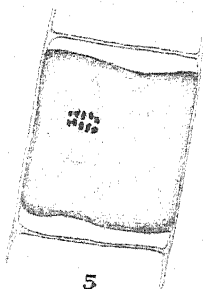
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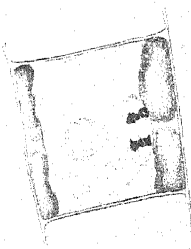
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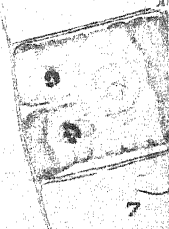
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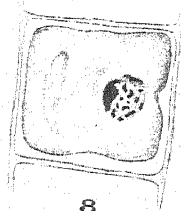
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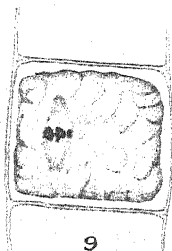
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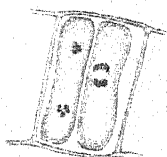
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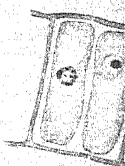
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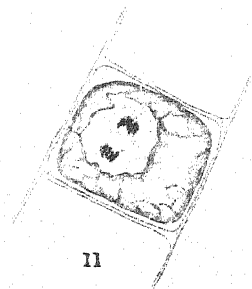


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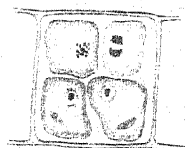


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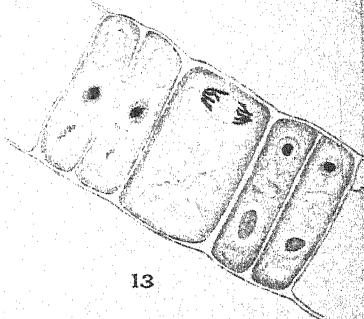
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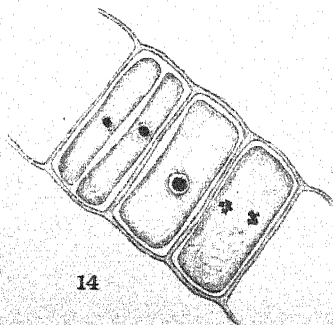
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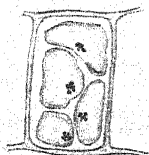
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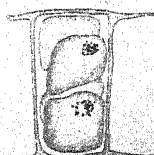
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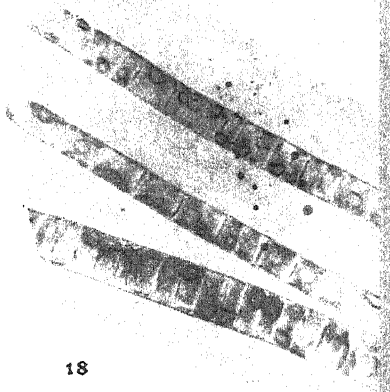




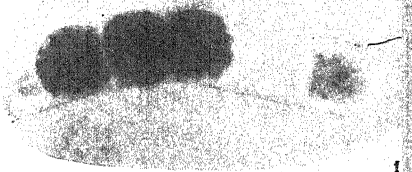
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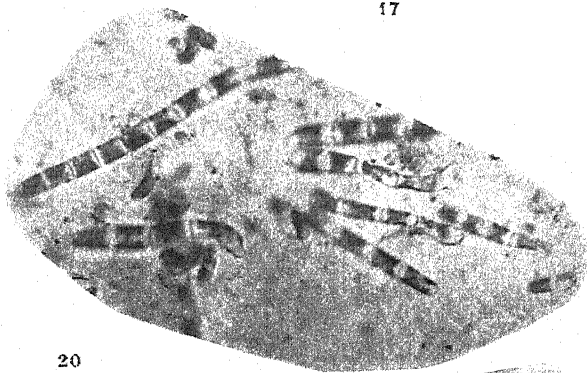
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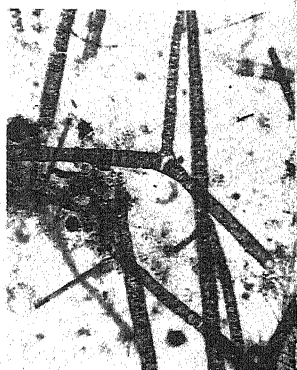
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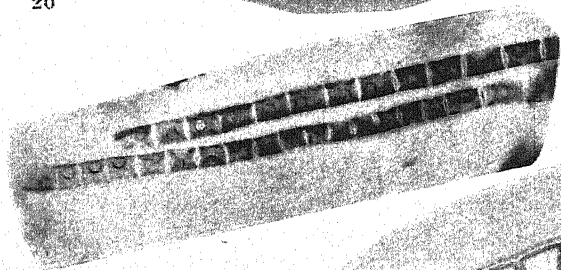
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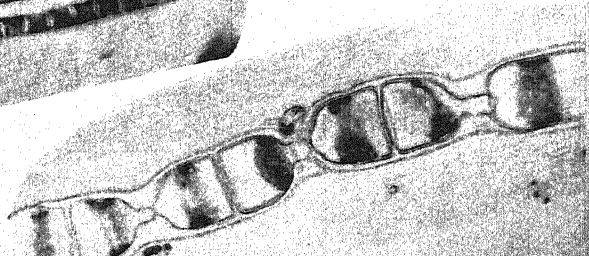
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Fig. 10. Stages in second division of nucleus. *a.* Anaphase and telophase. *b.* Prophase and resting nucleus.  $\times 1,250$ .

Fig. 11. Anaphase.  $\times 1,750$ .

Fig. 12. Stages in third division of nucleus.  $\times 1,250$ .

Figs. 13-15. Division of nucleus in spore-forming cell.

Fig. 13. Late anaphase and two completed divisions.  $\times 1,750$ .

Fig. 14. Resting nucleus, telophase, and one completed division.  $\times 1,750$ .

Fig. 15. *a.* Telophase of second division. *b.* Prophase of third division.  $\times 1,750$ .

PLATE XXVIII.

Figs. 16-18. *Ulothrix variata*.

Fig. 16. Photomicrograph of coiled gamete-forming filaments.  $\times 80$ .

Fig. 17. Release of gametes.  $\times 80$ .

Fig. 18. Photomicrograph of vegetative filaments.  $\times 320$ .

Figs. 19-22. *Ulothrix zonata*.

Fig. 19. Photomicrograph of cells containing microspores.  $\times 320$ .

Fig. 20. Photomicrograph of macro- and microsporelings after one week.  $\times 320$ .

Fig. 21. Photomicrograph of filaments arising from microspores after three weeks.  $\times 320$ .

Fig. 22. Photomicrograph of a branched filament showing rhizoid-like outgrowths of the cells and very thick wall. From material standing in a collecting tube.  $\times 500$ .

Fig. 23. Photomicrograph of a branched filament arising from a spore germinating on a slide in a tube of water.  $\times 80$ .



# Oidial Mycelia and the Diploidization Process in *Coprinus lagopus*.

BY

H. J. BRODIE, M.Sc. (Manitoba).<sup>1</sup>

## I. INTRODUCTION.

IN a previous paper the author (1) gave an account of the structure and function of the oidia of *Coprinus lagopus*. It was shown that: the oidia are produced in little masses in drops which crown the ends of aerial oidiophores; that the oidiophores are borne on haploid but never on diploid mycelia; that insects can transfer oidia of one sex to mycelia derived from spores of opposite sex; and that the oidia so transferred may germinate, fuse with the mycelia to which they have been brought, and cause these mycelia to become diploid. A haploid mycelium of basidiosporous origin, after having been diploidized through the agency of a haploid mycelium of oidial origin, has its nuclei in pairs, and these pairs of nuclei divide conjugately with the formation of clamp-connexions which can be readily observed with the microscope.

As already pointed out (1), a mycelium of *C. lagopus* derived from oidia (of one sex), which we may call an *oidial* mycelium, differs from a mycelium derived from a basidiospore, which we may call a *basidiosporous* mycelium, in the following points: (1) its hyphae are much thinner—only  $2-3\ \mu$  in diameter as against  $5\ \mu$  for the hyphae of a diploid mycelium; (2) it grows more slowly; (3) it produces no aerial hyphae except oidiophores on dung-agar media; (4) it does not produce any haploid fruit-bodies on horse-dung but remains sterile indefinitely; (5) it produces far more oidiophores per unit area of the culture medium; (6) it sometimes produces oidiophores beneath as well as above the surface of dung-agar culture media; and (7) the oidiophores are not infrequently branched.

While it was found by experiment that an oidial mycelium is able to diploidize a basidiosporous mycelium, the two following questions in respect to the part which oidial mycelia play in the diploidization process were left unsettled: (1) are *two oidial mycelia* of opposite sex able to

<sup>1</sup> Paper from the Department of Botany, University of Michigan, No. 370.

[Annals of Botany, Vol. XLVI. No. CLXXXIV. October, 1932.]

diploidize one another; and (2) is a *basidiosporous mycelium* of one sex able to diploidize an *oidial mycelium* of opposite sex? As a result of further work, now to be recorded, both these questions have been answered in the affirmative.

## II. MATERIAL AND METHODS.

Eleven haploid mycelia, Nos. 1–11, derived from as many basidiospores collected from a single fruit-body of *C. lagopus* obtained at Winnipeg, were isolated and transferred to stock-culture tubes of sterilized horse-dung. They were then paired in all possible ways and, with the help of the clamp-connexion criterion, they were sorted out into the usual four sexual groups as follows:

(*AB*) Nos. 1, 4, and 11.

(*ab*) Nos. 2, 5, 6, and 7.

(*Ab*) Nos. 3 and 10.

(*aB*) Nos. 8 and 9.

These eleven haploid basidiosporous mycelia all produced oidia in abundance. The oidia from mycelia Nos. 1, 3, 5, and 9 were sown on separate plates of dung-agar and thus four oidial mycelia were obtained.

To distinguish the eleven mycelia of basidiosporous origin from the four mycelia of oidial origin, the letter S will be set before any one of the former and the letter O before any one of the latter. Thus, S<sub>1</sub>, S<sub>3</sub>, S<sub>5</sub>, and S<sub>9</sub> indicate that mycelia Nos. 1, 3, 5, and 9 were each derived from a basidiospore, whereas O<sub>1</sub>, O<sub>3</sub>, O<sub>5</sub>, and O<sub>9</sub> indicate that these four mycelia were derived from oidia produced by mycelia S<sub>1</sub>, S<sub>3</sub>, S<sub>5</sub>, and S<sub>9</sub> respectively.

It was found that the oidia of *C. lagopus* germinate in far greater numbers on dung-agar cleared with white of egg than on uncleared agar. On this account cleared dung-agar was chosen as the culture medium for all the pairing experiments.

Two methods of pairing on dung-agar plates were employed: (1) the *equal-pairing* method in which the two haploid mycelia were equal in size, and were set side by side in the middle of the plate; and (2) the *unequal-pairing* method, introduced by Buller (2, 3), in which a pin-head mass of one haploid mycelium was set on the plate at the periphery of a large 6-cm.-wide haploid mycelium of opposite sex.

## III. THE PAIRING OF OIDIAL MYCELIA OF OPPOSITE SEX.

In the writer's first paper (1) two experiments were described in which oidia of opposite sex were sown together without the resultant mycelia becoming diploidized. The two sexual kinds of oidia were sown in hanging

drops of dung-agar. They germinated and the young mycelia fused together to form a single compound mycelium, but no clamp-connexions were developed. In reporting these experiments, it was remarked that 'further experiments are needed to decide whether or not the results of these two experiments are normal'. Further experiments in which oidia or oidial mycelia of opposite sex have been paired, not in hanging drops, but on plates where the mycelia could grow much more vigorously, have now been made with positive results in respect to diploidization, and these experiments will now be recorded.

The new experiments were made as follows: (1) oidia of opposite sex were sown in succession at the middle of a dung-agar plate, so that the oidia were mixed before they began to germinate; and (2) portions of ten-day-old oidial mycelia of opposite sex were paired on dung-agar plates by the equal-pairing method.

(1) The pairings of oidia were as follows:

oidia from S1 and S5 =  $(AB) \times (ab)$ ,

oidia from S3 and S8 =  $(Ab) \times (aB)$ ,

and both pairs were made in duplicate.

The mixed oidia in each of the four plates germinated in about twelve hours. The germ-tubes developed into young mycelia which soon intermingled and fused with one another. At the end of three days, as a result of this fusion, in each of the four plates a compound diploid mycelium was produced. Each of the four diploid mycelia, in the diameter of its hyphae, the non-production of oidiophores, the acute-angled mode of branching, and in the formation of clamp-connexions, exactly resembled a diploid mycelium produced by the mutual diploidization of two basidiosporous mycelia of opposite sex as observed by myself and illustrated by Buller (3).

(2) The pairings of the ten-day-old oidial mycelia were as follows:

mycelia O<sub>1</sub> and O<sub>5</sub> =  $(AB) \times (ab)$ ,

mycelia O<sub>3</sub> and O<sub>9</sub> =  $(Ab) \times (aB)$ ,

and both pairings were made in duplicate.

The paired mycelia in each of the four plates soon fused with one another and, three days after the pairings had been effected, in each of the four plates the two oidial mycelia had mutually diploidized one another. In four controls in which the four mycelia O<sub>1</sub>, O<sub>3</sub>, O<sub>5</sub>, and O<sub>9</sub> were grown separately on plates no diploidization took place, but each mycelium retained its haploid characters.

The diploid mycelium  $(AB) + (ab)^1$  produced by the mutual dip-

<sup>1</sup> For the introduction of the notation used here and in the next Section to distinguish between the two kinds of diploid mycelia in *C. lagopus* vide Buller (2, 3).

loidization of the  $O_1$  and  $O_5$  mycelia,  $(AB) \times (ab)$ , was transferred to sterile horse-dung in six wide tubes. At the end of ten to fourteen days the mycelium fruited, and the fruit-bodies were characteristically diploid in appearance (3, 4).

The results of the two series of experiments, (1) and (2), clearly prove that two oidial mycelia of opposite sex are able to diploidize one another.

#### IV. THE DIPLOIDIZATION OF AN OIDIAL MYCELIUM BY A BASIDIOSPOROUS MYCELIUM.

The unequal-pairing method was employed. An oidial mycelium was allowed to grow on a plate until it was 6 cm. in diameter, and then a pin-head mass of a basidiosporous mycelium of opposite sex was set by it at its periphery. The pairings were as follows:

small  $S_1$  and large  $O_5 = (AB) \times (ab)$ ,

small  $S_3$  and large  $O_9 = (Ab) \times (aB)$ ,

and both pairings were made in duplicate. The four mycelia  $S_1$ ,  $S_3$ ,  $O_5$ , and  $O_9$  were also set separately on plates, so that they might serve as controls.

About forty-eight hours after the pairings had been made, clamp-connexions began to appear at the junction of the two mycelia in each of the four plates and, in the course of three more days, the large haploid oidial mycelium in each of the four plates became completely diploidized as was indicated by the development of clamp-connexions on all its peripheral hyphae. The controls remained in the haploid condition.

The four experiments just recorded show that an oidial mycelium of one sex can be diploidized by a basidiosporous mycelium of opposite sex.

#### V. THE DIPLOIDIZATION OF A BASIDIOSPOROUS MYCELIUM BY AN OIDIAL MYCELIUM.

The unequal-pairing method was again employed. A basidiosporous mycelium was allowed to grow on a plate until it was 6 cm. in diameter, and then a pin-head mass of an oidial mycelium was set at its periphery. The pairings were as follows:

small  $O_5$  and large  $S_1 = (ab) \times (AB)$ ,

small  $O_9$  and large  $S_3 = (aB) \times (Ab)$ ,

small  $O_3$  and large  $S_9 = (Ab) \times (aB)$ ,

and all three pairings were made in duplicate. The six mycelia  $O_3$ ,  $O_5$ ,  $O_9$ ,  $S_1$ ,  $S_3$ , and  $S_9$  were also set separately on plates, so that they might serve as controls.

About forty-eight hours after the pairings had been made, clamp-connexions began to appear at the junction of the two mycelia in each of

the six plates and, in the course of three more days, the large haploid basidiosporous mycelium in each of the six plates became completely diploidized, as was indicated by the development of clamp-connexions on all its peripheral hyphae. The controls remained in the haploid condition.

The diploid mycelium  $(AB) + (ab)$  produced by the diploidization of the large  $S_1$  mycelium  $(AB)$  by the small  $O_5$  mycelium  $(ab)$  was transferred to sterile horse-dung in several wide tubes. In ten to fourteen days the mycelium fruited, and the fruit-bodies were characteristically diploid in appearance (3, 4). A similar result was obtained with a diploid mycelium  $(Ab) + (aB)$  produced by the diploidization of the large  $S_3$  mycelium  $(Ab)$  by the small  $O_9$  mycelium  $(aB)$ .

The results of the series of experiments just recorded show that a basidiosporous mycelium of one sex can be diploidized by an oidial mycelium of opposite sex, and they therefore confirm results of similar experiments described in the author's first paper (1).

#### VI. STERILITY OF HAPLOID OIDIAL MYCELIA.

Usually, when grown on horse-dung in a culture tube, a haploid basidiosporous mycelium of *C. lagopus* produces one or more haploid fruit-bodies (known by their paleness and more or less imperfect form) in two to three weeks from the time of inoculation. On the other hand, a haploid oidial mycelium appears to be incapable of fruiting. In 1931, the writer (1) grew an oidial mycelium on fresh sterilized horse-dung for three months without any sign of fruit-bodies being shown by it. Since then four oidial mycelia,  $O_1$ ,  $O_3$ ,  $O_5$ , and  $O_9$ , derived from oidia obtained from the four basidiosporous mycelia  $S_1$ ,  $S_3$ ,  $S_5$ , and  $S_9$ , have been grown on horse-dung for three months, and again the mycelia have remained completely sterile. On the other hand, the haploid mycelia  $S_1$ ,  $S_3$ ,  $S_5$ , and  $S_9$  all produced haploid fruit-bodies two to three weeks after they were set on dung in culture tubes.

The non-fruiting of haploid oidial mycelia may possibly be correlated with the great abundance of the oidiophores and oidia which these mycelia produce. Possibly they exhaust themselves in the production of oidia, and thus do not accumulate sufficient substance for the production of basidiosporous fruit-bodies.

#### VII. OIDIA OF *COPRINUS LAGOPUS* AND THE PYCNIOSPORES OF THE RUST FUNGI.

In the writer's previous communication (1) it was pointed out that the oidia of *C. lagopus* and the pycniospores of the Rust Fungi resemble one another in: (1) their early appearance on haploid mycelia; (2) in their being immersed in a fluid which is attractive to flies; (3) in their

transportation by flies from one mycelium to another ; and (4) in their ability to bring about the diploidization of mycelia of basidiosporous origin and of opposite sex ; and it was also suggested that, if oidial mycelia are not able to diploidize oidial mycelia of opposite sex but only basidiosporous mycelia of opposite sex, then the oidia of *C. lagopus* resemble the pycniospores of the Rust Fungi in one further particular. Since, in this paper, it has been shown that in *C. lagopus* oidial mycelia of opposite sex *are* able to diploidize one another, the supposed resemblance of oidia to pycniospores based on the limitation of diploidizing power must now be regarded as having no basis in fact.

The experimental work recorded above was carried out in the Department of Botany of the University of Michigan. To Professor A. H. R. Buller, of the University of Manitoba, I am indebted for stimulating suggestions and for the revision of the manuscript, and to Dr. E. B. Mains, of the University of Michigan, I desire to extend my thanks for his interest in the work and for various criticisms.

#### VIII. SUMMARY.

1. The oidia of *C. lagopus* germinate better on cleared dung-agar than on uncleared.
2. Oidial mycelia (mycelia derived from oidia) grow indefinitely on sterile horse-dung without producing any haploid fruit-bodies.
3. Two oidial mycelia of opposite sex are able to diploidize one another.
4. An oidial mycelium of one sex can be diploidized by a basidiosporous mycelium of opposite sex.
5. A basidiosporous mycelium of one sex can be diploidized by an oidial mycelium of opposite sex.

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## The British Coal Measure Floras.

BY

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**D**R. R. KIDSTON (21), in his Presidential Address to the Royal Physical Society of Edinburgh, described the four plant assemblages which he recognized as characteristic of the Upper, Transition, Middle, and Lower Coal Measures, and which, in 1905, he (22) designated the Radstockian, Staffordian, Westphalian (Yorkian), and Lanarkian floras, respectively.

It was Kidston's intention to bring up to date these important assemblages, but the work was delayed until his great Memoir on the 'Fossil Plants of the Carboniferous Rocks of Great Britain' (24) should be completed. Most unfortunately, the Memoir itself was left but half finished.

The writer has therefore undertaken the work. This consisted of three parts: (i) the inclusion of many recent records, and the extension of the known vertical range of a number of species, (ii) the elimination or correction of unsatisfactory records, and (iii) a critical revision of the Yorkian and Lanarkian floras (9).

Kidston's Radstockian and Staffordian divisions of the Coal Measures correspond with those based on animal remains, and have received general acceptance. His Yorkian and Lanarkian divisions, on the other hand, were less satisfactory, and certain apparent discrepancies appeared in Coal Measure stratigraphy when floral results were compared with faunal determinations. Criticism (11, 19, 27, 28) has mainly been directed to these two divisions.

It has recently been shown (9) that a re-examination of Kidston's own records from the two lower divisions brought to light two facts: (i) that his dividing line between them was unsound; i.e. that a portion of his Yorkian in Yorkshire was equivalent to part of his Lanarkian in Scotland, a conclusion which receives support from faunal evidence, and (ii) that when the dividing line is taken at a lower level, a flora is revealed which contains distinctive elements. In other words, it is at the top of the lower portion of the Coal Measures that a definite change occurs in the flora (at the Mill or Auchingane Coal in Stirlingshire and the Better Bed<sup>1</sup> Coal in Yorkshire). To this lowest portion of the Coal Measures the term Lanarkian must now be applied.

<sup>1</sup> D. A. Wray (in MS.).

It should here be observed that Kidston strongly suspected the presence of a Lanarkian flora in the Lower Coal Measures of South Wales. He did not, however, survive to see a representative assemblage of species from those beds. During the geological revision, by Dr. Thomas Robertson, of the area around Merthyr Tydfil, the writer, who was concerned with the palaeobotany of the Carboniferous, had the great advantage of assistance from, and collecting by, Mr. W. D. Ware of Ystradgynlais, near Swansea. Mr. Ware brought to light a flora, which included a number of the chief species recorded by Kidston from the lowest Coal Measures of Scotland, which shows a general correspondence to those known from certain Continental coalfields (29). Miss Emily Dix (11, 12, 13) has arrived at similar results.

Kidston cited a large assemblage of Yorkian plants, many species of *Sphenopteris*, *Neuropteris*, *Sigillaria*, &c., being of common occurrence, but records from his Lanarkian included many Yorkian forms, though a species which was common to both divisions was usually markedly rarer in the lower. Although he observed that a number of species were restricted to his Lanarkian, he placed little stratigraphical reliance on these forms. There were thus two distinguishing features of the Lanarkian: (i) the absence or rarity of the majority of Yorkian forms, and (ii) the presence of certain restricted species. These criteria will now be examined, first dealing with the restricted plants.

The following species were described in 1894 (a) as 'apparently peculiar to' the Lower Coal Measures: *Sphen. adiantoides*<sup>1, A</sup>, *Sphyropteris boehmischii*<sup>2</sup>, *Neuropteris rectinervis*<sup>A</sup>, *N. blissi*<sup>A, 2</sup>, *N. crenulata*<sup>A, 2</sup>, *Stachannularia northumbriana*<sup>3</sup>, *Lepidodendron serpentigerum*<sup>A, 3</sup>, *Sigillaria walchi*<sup>A</sup> and *Psygmyphyllum flabellatum*<sup>A</sup>. A glance at the complete list of species given (21, pp. 238–57), shows that, though he probably regarded them as less characteristic, the following were also at that time known from the Lanarkian only: *Sphenopteris schillingsi* Kidston (non Andrae)<sup>4</sup>, *S. amoena*, *Renaultia microcarpa*<sup>A</sup>, *R. gracilis*<sup>A</sup>, *Cyclothea biseriata*<sup>6, 2</sup>, *Dactylothea caudata*<sup>7</sup>, *Megaphyton approximatum*<sup>A</sup>, *Lepidodendron peachi*<sup>A</sup>, *L. fusiforme*<sup>A</sup>, *L. jaraczewski*<sup>A</sup>, *Lepidostrobis squarrosus*, *Sigillaria lenti-*

<sup>1</sup> *Sphenopteris adiantoides* is now referred to the genus *Adiantites*.

<sup>A</sup> This species has since been recorded from measures above Kidston's Lanarkian.

<sup>2</sup> Although Kidston (24, p. 362) subsequently dealt with the British representatives of the genus *Sphyropteris*, he omitted mention of this species. The record must, therefore, be suppressed.

<sup>3</sup> *Lepidodendron serpentigerum* Koenig, = *L. distans* Lesquereux.

<sup>4</sup> *S. schillingsi*, Kidston (non Andrae) = *Adiantites tenellus*.

<sup>5</sup> *S. amoena*, Kidston = *S. amoenaeformis*.

<sup>6</sup> The Kiltongue Coal, from which this record was made, falls into the Yorkian Series on the new classification. The species is very rare, and has been found at one locality only.

<sup>7</sup> The beds from which these forms came now fall within the Yorkian.

<sup>8</sup> *D. caudata* was omitted from Kidston's treatment of the genus in 1923–5 (24, p. 382), so that the record is of no value.

cularis, *S. orbicularis*<sup>A</sup>, *Cardiocarpus orbicularis*<sup>A, 1</sup>, and *Psilotites unilateralis*<sup>A</sup>.

It will be seen, from the above, that out of the 23 species originally listed as being found in the Lower Coal Measures only, no less than 16 are now known from higher measures, while two other records must be suppressed entirely.

*Sphenopteris schillingsi*, Kidston (non Andrae) = *Adiantites tenellus*, *A. adiantoides*, *Neuropteris rectinervis*, *Lepidostrobus squarrosus*, and *Lepidodendron peachi* are among the Lanarkian forms at present recognized, the most characteristic being *N. rectinervis* (= *N. schlechani*). *Stachannularia northumbriana* is a rare cone, while Sauveur's *Sigillaria lenticularis* is not very satisfactory. It should be compared with Lesquereux's *S. leptoderma*.

*Sphenopteris hoeninghausi*, *Sigillaria elegans*, and *Lepidophloios acerosus* were recorded by Kidston in 1894 from both the Middle and Lower Coal Measures. Unfortunately, he did not indicate their relative frequency.

The writer recently (10) published a list of 20 species which are, so far as is at present known, restricted in distribution to Kidston's Lanarkian, but at the same time observed that 17 of these are confined to the Lanarkian of Scotland (those marked with a star), and must, at least for the present, be regarded as local forms, having little or no positive correlative value. This is further emphasized by the fact that 18 of the species are of very rare occurrence, in many cases there being a single record (marked † in the list). The twenty restricted plants are as follows: *Adiantites tenellus*, Kidston \* †, *Cyclothea biseriata*, Kidston †, *Renaultia germanica*, Potonie \* †, *Rhodea sparsa*, Kidston \* †, *Sphenopteris amoenaformis*, Kidston \* †, *S. arberi*, Kidston \* †, *S. bacumleri*, Andrae, *S. formosa*, Gutbier \* †, *S. hulseni*, Gothan \* †, *S. lanarkiana*, Kidston \*, *S. quadriloba*, Kidston \* †, *Telangium digitatum*, Kidston \* †, *Eupecopteris minor*, Kidston \* †, *Calamites ohlsbachensis*, Sterzel †, *Sigillaria incerta*, Kidston \* †, *S. latibasa*, Kidston \* †, *S. lenticularis*, Sauveur \* †, *S. strivelsensis*, Kidston \* †, *Lepidostrobus squarrosus*, Kidston \* †, and *Trigonocarpus schultzeanus*, Goepfert & Berger †.

It is, therefore, not surprising that Kidston's chief distinguishing criterion between the Lanarkian and the Yorkian was a negative one. Though the Yorkian possessed many characteristic species, some few of these occurred also in the Lanarkian, but the vast majority were absent from the lower division. This necessarily led to several doubtful, even erroneous, correlations. Thus, Kidston's correlation of part of the Claverley Borehole with his Lanarkian, on purely negative evidence (23) cannot be upheld, as the writer (10) has shown. He (25) similarly referred the most northerly British patch of Coal Measures, in the neighbourhood of Inninmore, Sound of Mull, to the Lower Coal Measures on insufficient

<sup>A</sup> This species has since been recorded from measures above Kidston's Lanarkian.

<sup>1</sup> Now referred to the genus *Samaropsis*.

evidence. The late Dr. E. A. N. Arber (1, 2) was also led to refer the barren Coal Measures of Devon to the Middle Coal Measures on inconclusive plant evidence.<sup>1</sup>

With the classification recently proposed we have, on the other hand, a definite assemblage, and the component elements can be grouped under three headings: (i) common and characteristic species, (ii) subsidiary forms, and (iii) apparently local plants. These are set forth in the following Table:

#### THE LANARKIAN FLORA

Chief Species.	Subsidiary Species.	Local Forms.
<i>Neuropteris rectinervis</i> (Kidston) (= <i>N. schiehani</i> , Stur)	<i>Adiantites adiantoides</i> (L. & H. non Schlotheim)	<i>Adiantites tenellus</i> , Kidston
<i>Mariopteris acuta</i> , Brongniart	<i>A. bondi</i> , Kidston <sup>2</sup>	<i>Lepidodendron peachi</i> , Kidston
<i>Sphenopteris hulseni</i> , Gothan		<i>Sigillaria incerta</i> , Kidston
<i>S. bacumleri</i> , Andrae		<i>S. strivelsenensis</i> , Kid- ston
<i>S. hoeninghausi</i> , Brongniart		
<i>Sphenophyllum amplum</i> , Kidston		
<i>Sigillaria elegans</i> , Brongniart		
<i>Lepidophloios acerosus</i> , L. & H.		

The Yorkian and Lanarkian floras are now sufficiently distinct for ready recognition. There are, however, still a number of species which occur in both divisions, and the negative feature which distinguished Kidston's Lanarkian still obtains. Whereas, however, it was of the first importance in the diagnosis of Kidston's Lanarkian,<sup>3</sup> it now takes a place subordinate to the positive elements recognized in the flora.

Of plants common to the two divisions, the following are more frequent in the lower: *Adiantites adiantoides*, *Neuropteris rectinervis*, *Sphenopteris hoeninghausi*, *Sigillaria elegans*, and (?) *Lepidophloios acerosus*. The remainder, with the exception of *Lepidodendron aculeatum*,<sup>4</sup> are of more frequent occurrence in the Yorkian. This applies to *Alethopteris lonchitica*, *Urnatopteris tenella*, *Sphenopteris schatzlarensis*, *S. laurenti*, *S. nummularia*, *S. spiniformis*, *Zeilleria delicatula*, *Z. hymenophylloides*, *Myriotheca anglica*, *Lepidophloios laricinus*, *Bothrodendron minutifolium*, *Sigillaria micaudi*, *S. scutellata*, *S. tessellata*, and *Lepidodendron lycopodioides*. Roots (*Stig-*

<sup>1</sup> Crookall, R., *The Plant Horizons Represented in the Barren Coal Measures of Devon, &c.* *Proc. Cotteswold Nat. F. C.*, vol. xxiv, 1930, pp. 27-34.

<sup>2</sup> Only two specimens of *Adiantites bondi* are known, the first described by Kidston (24, Pl. XLIV, Fig. 5, Pl. XLVII, Fig. 2, p. 192) from a blue bind below the Black Bed Coal at the Leeds Patent Brickworks Quarry, Dolly Lane, Leeds, and the second identified by the writer from a collection of plants recently made from the Coal Measure of Inimore, Sound of Mull. In each case the horizon is open to some doubt. The Mull plants were collected by Dr. Murray Macgregor. It is hoped to obtain further specimens.

<sup>3</sup> Kidston (21, p. 225) observed that it was not so much on the presence of the species he cited as restricted to the Lower Coal Measures that he relied, 'as on the absence of so many species that are common in the Middle Coal Measures.'

<sup>4</sup> This species is about equally common in the two divisions.

*maria*, *Pinnularia*, and *Radicites*), cones (*Lepidostrobus Sigillariostrobus*, &c.), bracts (*Lepidophyllum*) as well as other forms of limited stratigraphical utility are omitted from these lists. The writer is shortly publishing an account of the value of various plants in Coal Measure stratigraphy, and the relative importance of each form will there be assessed.

We are now in a position to indicate the chief elements in the floras as at present recognized. While many of these were described and figured in Kidston's 'Fossil Plants of the Carboniferous Rocks of Great Britain' (24), the parts of this work published to date deal only with a portion of the Pteridosperms and Ferns, while the Lycopods, Sphenophylls, Equisetales, and Cordaitales remain to be treated. Most of the species, however, have been figured, and briefly described in the writer's 'Coal Measure Plants' (5).

Before giving the floras, it may be observed that the Staffordian Series contains a plant assemblage, which is transitional between those of the Radstockian and Yorkian. Although the flora is a 'mixed' one, certain Radstockian forms are very rarely, if ever, found, while the same is true of a number of Yorkian species. It is too early, however, to give a list of plants from the divisions above and below which do not occur in the Staffordian. The most characteristic Staffordian species can, nevertheless, be indicated. They are as follows: *Linopteris münsteri* (Eichwald), *L. obliqua* (Bunbury), *Neuropteris tenuifolia*, (Schlotheim),<sup>1</sup> *N. ovata*, Hoffmann, *N. flexuosa*, Sternberg, *N. rarinervis*, Bunbury, *N. scheuchzeri*, Hoffmann, *N. pseudo gigantea*, Sternberg, *Alethopteris serli-grandini* (Brongniart), *Mariopteris nervosa* (Brongniart), *M. latifolia* (Brongniart), *Sphenopteris striata*, Gothan, *S. neuropteroides* (Boulay), *Sphenophyllum emarginatum* (Brongniart), and *S. majus* (Bronn) (4, 6, 14).

Some observations are necessary on the various sub-divisions of the Staffordian Series and, for the sake of clearness, these are best made in historical sequence.

The Staffordian Series (Transition Coal Measures) was originally recognized in 1894 by Kidston (21) in South Wales (the Lower Pennant Rocks), and in Somerset (the New Rock and Vobster Series), and, in the same year, he (20) observed, 'Such Transition Series are very rare in the Carboniferous of Britain, and in other coalfields . . . the different divisions are more sharply marked off'. Since then, however, the division has been recognized in Cumberland, Lancashire, North Wales, Warwickshire, Shrewsbury, Forest of Wyre, Forest of Dean (7) and Kent, while it very probably occurs in the Coal Measures of Ayrshire (8) and of Canonbie.<sup>2</sup>

In 1902, Dr. Walcot Gibson (15, 16, 17) divided the Coal Measures

<sup>1</sup> In the basal beds.

<sup>2</sup> Since, in 1930, the writer suggested that Staffordian rocks might be present in the lower part of the Red Measures of Ayrshire, faunal evidence in support has been adduced by Dr. J. Pringle and Mr. W. Manson (26).

## THE COAL MEASURE FLORAS.

(Chief Species are marked by an asterisk.)

## RADSTOCKIAN.

(Upper Coal Measures).

PTERIDOSPERMS AND  
FERNS  
(A) Sphenopterideae*Sphenopteris macilentia*\*  
*S. neuropteroides*\*

## YORKIAN

(Middle Coal Measures).

*Corynepteris coralloides*  
*Diplomema furcatum*\*  
*Renssalia rotundifolia*  
*R. gracilis*  
*Senslenbergia ophiodermatica*  
*Sphenopteris dilatata*\*  
*S. laurenti*\*  
*S. sauveuri*\*  
*S. schatzlarenensis*  
*S. spiniformis*  
*S. striata*\*  
*S. trifoliolata*\*  
*S. trigonophylla*\*  
*S. murrai*  
*S. footheri*  
*S. mummularia*  
*Muriopteris nervosa*\*  
*Corynepteris sternbergi*\**Acitheca polymorpha*\*  
*Asterotheca arborescens*\*  
*A. cyathica*\*  
*A. oreopteridia*\*  
*Ptychocarpus unius*\*  
*Enpecopteris camerunensis*  
*E. jettii*

## (B) Pecopterideae

## LANARKIAN

(Lower Coal Measures).

*Adiantites adiantoides*  
*A. tenellus*  
*Mariopteris acuta*\*  
*Sphenopteris hoeninghausi*\*  
*S. bacouleri*  
*S. hulseni*

## (C) Alethopterideae

*Alethopteris senli-graudinii*\*

*Alethopteris decurrens*\*  
*A. lonchitica*\*  
*A. integra*\*  
*A. valida*\*  
*Lonchopteris rugosa*\*

*A. lonchitica*

## (D) Neuropterideae

*Neuropteris ovata*\*  
*N. flexuosa*\*  
*N. macrophylla*\*  
*N. rutilans*  
*N. schuchzeri*  
*Odontopteris lindleyana*\*

*Neuropteris heterophylla*\*  
*N. gigantea*\*  
*N. tenuifolia*\*  
*N. obliqua*\*  
*N. grangeri*  
*N. microphylla*  
*N. blatti*  
*N. crenulata*  
*L. neuropteroides*

*Neuropteris schlehmani*  
 (= *N. rectinervis*)

## SPHENOPHYLLALES

*Sphenophyllum emarginatum*\*

*Sphenophyllum cuneifolium*\*  
*S. saxifragae-folium*\*  
*S. myriophyllum*\*  
*S. trichomanosum*\*  
*S. majus*

*S. amplum*

## EQUISETALES

*Annularia stellata*\*  
*A. sphenophyllioides*

*Annularia rudata*†

*A. dubia*  
*Calanites carinatus*†  
*C. cisti*†  
*C. schutzeiformis*  
*C. undulatus*†  
*C. goepperti*†  
*C. sachsei*†  
*C. fulaceus*  
*Asterophyllites charaeformis*†

Most of the Yorkian forms  
 also occur in the Lanarkian.  
 (Those marked †.)

THE COAL MEASURE FLORAS (continued).

LYCOPODIALES	RADSTOCKIAN (Upper Coal Measures).	YORKIAN (Middle Coal Measures).	LANARKIAN (Lower Coal Measures).
	<i>Lepidodendron wortheni</i> <i>Sigillaria cumulata</i> *	<i>Lepidodendron aculeatum</i> <i>L. acutum</i> <i>L. lycopodioides</i> * <i>L. obovatum</i> * <i>L. ophiurus</i> * <i>L. samile</i> <i>L. loricatum</i> <i>L. sub-wortheni</i> <i>L. distans</i> <i>L. jaraczewski</i> <i>L. rimosum</i> <i>Sigillaria discophora</i> * <i>S. laevigata</i> <i>S. mammillaris</i> <i>S. rugosa</i> * <i>S. tessellata</i> <i>S. elongata</i> * <i>S. principis</i> * <i>S. scutellata</i> * <i>S. boblayi</i> <i>S. schlotheimi</i> <i>Lepidophloia acerosus</i> <i>L. laricinus</i> * <i>Bothrodendron minutifolium</i> <i>B. punctatum</i> *	<i>Lepidodendron psacchi</i> <i>Sigillaria elegans</i> * <i>S. incerta</i> <i>S. micaudi</i> <i>S. struelensis</i> <i>Lepidophloia acerosus</i> * <i>L. laricinus</i>
	<i>Pecordites microstachys</i> * <i>Cordites angulosostratus</i> *	<i>Cordites principalis</i> <i>C. borassifolius</i> * <i>Dorycordites palmaeformis</i> *	<i>Cordites principalis</i>
CORDAITALES			



of North Staffordshire lying above the Bassey Mine Ironstone into the Blackband Group, the Etruria Marl Group, the Newcastle-under-Lyme Group and the Keele Group. The floras of these divisions were described by Kidston (22), while Arber (3) recorded fifty-eight plant species from the 'Etruria Marl Group' of South Staffordshire. In North Staffordshire the Etruria Marls had proved practically barren. Kidston correlated the Keele Group with the lower portion of his Radstockian Series.

In the list below is given a comparison of the palaeobotanical divisions recognized in Scotland, Yorkshire, and elsewhere.

#### PALÆOBOTANICAL DIVISIONS

Area.	Division.	Kidston's Results.	Revised Results.
SCOTLAND	Yorkian	From a short distance above Skipsey's Marine Band.	Do.
	Lanarkian	Below Ell Coal of Lanarkshire.	Below Auchingane Coal of Stirling.
YORKSHIRE	Yorkian	Below Shafton Marine Band <sup>1</sup>	Do.
	Lanarkian	Below Better Bed Coal	Do.
S. WALES	Yorkian	Below No. 3 Rhondda Seam (= Lower Pinchin).	Do.
	Lanarkian	Unrecognized.	Below Cnapiog Seam.
LANCASHIRE	Yorkian	Below Ardwick Group	Do.
	Lanarkian	Below Arley Mine	Do.

Dr. Wheeler Hind (18), in the discussion following Kidston's paper (22) pointed out (i) that a similar flora and fauna occurred both above and below the Bassey Mine, which was, therefore, unsuitable as the base of a sub-division, (ii) he had made his sub-division at the horizon of the Gubbin Ironstone, where *Anthracomya phillipsi* came in for the first time, on lithological grounds. Dr. Gibson has also subsequently suggested that the base of the Blackband Group might be drawn at the Cannel Row Coal, some 300 ft. below the Bassey. Miss E. Dix (14) has confirmed Dr. Hind's observation, and traced the presence of the 'mixed' (Staffordian) flora to just below the Chalky Mine Ironstone, about 400 ft. below the Bassey Mine. The evidence, both floral and faunal, indicates that the upper portion of the Middle Coal Measures (of Kidston) in North Staffordshire must be classified with the Staffordian Series, a course which has lithological support. As Miss Dix (14) observes, this affects a palaeobotanical correlation of beds in the Transition Coal Measures of other coalfields with the Blackband, Etruria Marl, and Newcastle-under-Lyme Groups of North Staffordshire, necessitating revision of the Staffordian floral sub-divisions.

<sup>1</sup> D. A. Wray (in MS.).

The Yorkian Series of Yorkshire may be sub-divided at the Silkstone Coal into a Lower and an Upper division, the Lower having an abundance of *Alethopteris lonchitica* and *Neuropteris gigantea* (typical form). *N. obliqua* also occurs commonly in this division, though it has a more extended range. Specimens of *N. schlehani* and other Lanarkian forms may occasionally be found in the Lower but are absent from the Upper Yorkian. The Upper Yorkian is characterized by the maximum development of *Neuropteris tenuifolia*, *Sphenophyllum myriophyllum* and *Neuropteris pseudogigantea*. *Lonchopteris bricei*, though rare, is confined to this division, while *Neuropteris grangeri*, *N. microphylla*, *Sigillaria elongata* and *S. scutellata* here attain their maximum. *Sphenophyllum majus* is present and is unknown from the Lower Yorkian. *Alethopteris davreuxi* may also prove to be a form characteristic of the Upper Yorkian.

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# Some Fossil Dicotyledonous Woods from the Miocene (?) Beds of East Africa.

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With Plate XXIX and four Figures in the Text.

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## I. INTRODUCTION.

THE following is a description of certain fossil dicotyledonous woods, which were obtained by Dr. Felix Oswald from the uppermost (grey clay) beds of the Miocene (see postscript) series of Kenya Colony (24). All the specimens of this collection come from Kikongo, near Karungu (S.  $0^{\circ} 50'$  by E.  $34^{\circ} 10'$ ) on the eastern shore of Lake Victoria Nyanza. They consist of mineralized portions of tree-trunks ranging up to 1 ft. in diameter, and to  $1\frac{1}{2}$  ft. in length. The infiltrated material is mainly calcite, but crystals of various other substances occur,<sup>1</sup> and in many cases these crystals almost completely obscure the structure of the wood; in four cases, however, sufficient detail may be traced to allow of description, and also of a certain degree of comparison with recent and other fossil types.

A discussion of the problems connected with the identification of fossil woods, and an explanation of the nomenclature employed by the writer for such material, appeared in this Journal in April, 1932 (1).

The specimens here described are the property of the Geological Department of the British Museum (Natural History); reference is made to them in the descriptions under their museum registration numbers.

<sup>1</sup> Mr. R. C. Spiller, of the Mineral Department, University of Oxford, hopes shortly to publish a description of these crystals.

II. DESCRIPTIONS AND COMPARISONS.<sup>1</sup>Specimen v. 21360 (Sections *a* and *c*).<sup>2</sup>1. *Description* (Text-fig. 1, *a* and *b*; Pl. XXIX, Fig. 1).

Specimen v. 21360 is an imperfectly preserved wood in which seasonal zoning is not marked. The structure as seen in transverse section is definitely characterized by continuous, concentrically-arranged bands of xylem parenchyma (Pl. XXIX, Fig. 1; Text-fig. 1*a*); these bands are very slightly undulated, and are of fairly even width, comprising from 5 to 8 (usually 5 or 6) radially-arranged elements. The intervening areas are occupied by thick-walled fibres, in bands of (apparently) from 7 or 8 to 12 cells in width. The outlines of the fibres are very indistinct, and the fibre bands are therefore indicated in Text-fig. 1*a* only by shading; the fibres do not appear to be quite so regularly arranged in radial series as the parenchyma cells, but the clue to the number of cells in a subradial row is given by the presence of parenchyma cells accompanying an occasional ray on either side (Text-fig. 1*a*, *r''*). In tangential section the fibres and parenchyma may be distinguished (Text-fig. 1*b*), though the minute structure of their walls, such as pitting, is obscure. The fibres are elongated and pointed, while the parenchyma cells are short, with horizontal or inclined end walls; there is no indication of tier-like, or storied, arrangement of the parenchyma cells (Text-fig. 1*b*).

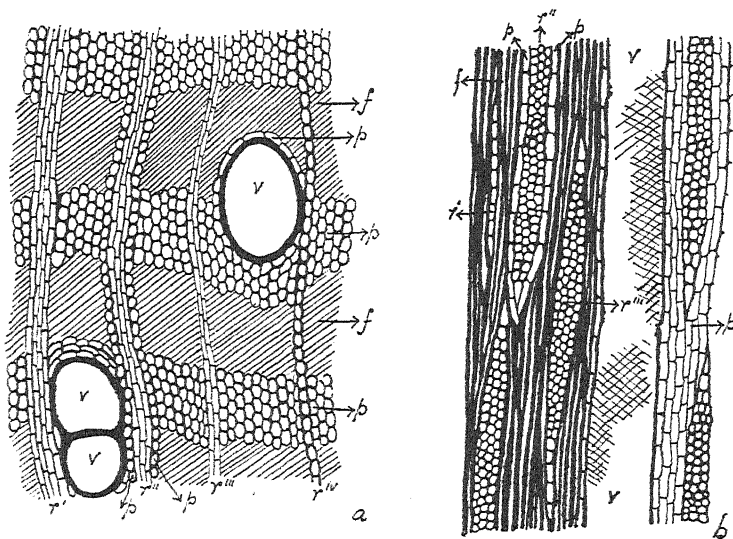
The wood is diffuse-porous, and practically isoporous, the vessels being thin-walled and fairly large (Pl. XXIX, Fig. 1); they are scattered, often deflecting the rays, and are only partially enclosed in the parenchyma bands; where they abut on the fibre bands, however, they are typically surrounded by one or two layers of parenchyma cells. Usually they are isolated or in pairs (Text-fig. 1*a*), less frequently in short radial rows of from 3 to 5, or in groups of 3; an average size of the isolated vessels is 270  $\mu$  (radial diameter) by 190  $\mu$  (tangential diameter). In a few cases there are evidences of tyloses. As seen in longitudinal section, the individual vessels appear to be rather short, and completely perforate at their ends; their limits are, however, very difficult to determine, owing to cracks in the petrifying material. Here and there are indications that the vessel-walls possess a close network of small pits, but it is impossible to say whether the pits are simple or bordered.

The rays are not very strongly marked, as seen in transverse section; they are from one to four cells wide (Text-fig. 1*a*), the cells being narrow and radially elongated in the case of the multiseriate, and more rounded in

<sup>1</sup> The descriptions of these fossil woods are not 'Linnean' (cf. Moll and Janssonius, 23; also Moll, 21, p. 38); the preservation of the material is such that many data are lacking, and it therefore seems advisable to give connected, rather than schematic, descriptions of the specimens.

<sup>2</sup> In all the specimens described, *a*, *b*, and *c* refer to the transverse, radial, and tangential sections respectively; only the sections which provide structural data are registered.

the uniseriate examples. The rays sometimes widen a little where they run through a parenchyma band (Text-fig. 1*a*, *r'*), and occasionally a ray is accompanied by parenchyma cells where it traverses a fibre-band



TEXT-FIG. 1. v. 21360. *a*. Diagram showing a portion of the transverse section, with alternating bands of fibres (*f.*) and parenchyma (*p.*), vessels (*v.*), and heterogeneous rays (*r'*, &c.); *r''* is accompanied by ordinary parenchyma cells on either side even where it passes through a fibre zone (cf. *r'''* in diagram *b*). *b*. A portion of a tangential section, showing fibres (*f.*), a vessel (*v.*), parenchyma (*p.*), and the heterogeneous rays (*r'*, &c.); *r'* is a shallow uniseriate ray; *r''* is accompanied by parenchyma (cf. *r'''* in diagram *a*); and *r'''* shows the typical depth of a multiseriate ray. The cross-hatching of the vessel indicates the close network of pits. (The magnification of these diagrams is 60 diameters; their main outlines were made with the aid of a camera lucida, but owing to the poor preservation of the material, the detail is represented in a purely diagrammatic manner; in *a* the fibres are indicated only by shading as their outlines are very indistinct.)

(Text-fig. 1*a* and *b*, *r''*). The preservation of the material is not sufficiently good to show the pitting of the cell-walls. The rays are fairly numerous, being separated by (typically) from 5 to 9 rows of the small radially-arranged elements of the wood parenchyma. In tangential section (Text-fig. 1*b*) they are seen to be scattered, without any definite arrangement; they are very variable in extent, being from 9 or 10 (in the case of the comparatively infrequent uniseriate rays) to 35 or 40 cells in height. In tangential view of the wood the majority of the ray-cells are rounded in section; the cells at the upper and lower edges of the rays are, however, elongated, and are also somewhat larger than the others, so that the rays are 'heterogeneous', though not markedly so (Text-fig. 1*b*).

## 2. Comparison with recent and other fossil types.

In searching amongst recent timbers for structures similar to those displayed by the East African fossil woods, the extensive series of sections

and photographs in the Imperial Forestry Institute, Oxford, and the material and photographs in the Cambridge School of Forestry, have been examined. Reference has also been made to various works on recent timbers and wood-structures, notably to those of Boulger (4), von Brehmer (5), Gamble (15), Hopkinson (17), Jones (18), Lecomte (20), Moll and Janssonius (22), Perrot (25), Record and Mell (26), Solereder (31), and Stone (32); also to Weale's series of microphotographs of woods in the Botanical Department of the British Museum (38).

Owing to lack of sufficient structural data due to imperfect fossilization of the material, and of any information concerning associated remains of vegetative and reproductive organs, it is not possible to do more than compare these East African fossils—which may, or may not, represent existing genera—with recent, and other fossil, timbers; and, in the very incomplete state of knowledge with regard to the essential diagnostic characters of woods, it must be realized that these comparisons are themselves somewhat tentative, for the material of any one type available for examination was limited, and might not necessarily give an idea of the really representative structure of the timber (cf. 1, pp. 361, 362). Moreover, although a large number of recent timbers was examined, these represent only a small portion of the woody Dicotyledons; and further researches may reveal types more similar to the fossils than those with which comparisons are here made.

Specimen v. 21360, with its alternating zones of fibres and parenchyma, is a wood of the *Ficus*-type, which is widely distributed amongst the families of Dicotyledons (1, p. 359, Table III); an initial comparison with *Ficus* is therefore indicated. *Ficus* is a large genus, containing 700 species, and only a very few of these were obtainable for examination; Gamble, however, notes that the wood structure throughout the genus is very uniform (15, p. 637), and such species as were available, for example, *F. Bengalensis*, showed considerably wider rays, much more markedly swollen in the parenchyma zones, than is the case in v. 21360. Moreover, storied arrangement of xylem parenchyma occurs in *Ficus*, though it is somewhat sporadic; while there is no trace of storied structure in v. 21360. *Ficus* also possesses crystal-cells; this is a point, however, in which no comparison is possible, owing to the state of preservation of the fossil.

In the original notes on these fossil woods, when only transverse sections were available, a comparison was made between v. 21360 and certain Leguminosae of the sub-family Papilionatae (1, p. 357, footnote 1). It is true that many of the Leguminosae possess the *Ficus*-type of structure; Gamble's description of the 'Dalbergia group', for example, as having narrow concentric bands of parenchyma and rather scanty vessels, usually independent of the parenchyma bands, immediately suggests a comparison between v. 21360 and members of this group. An examina-



tion of sections of various representatives, however, reveals differences in detail—for example, the uniformly uniseriate rays and marked storied structure in *Pterocarpus*—which show that any similarity of type is mainly superficial.

Of other Leguminosae, *Pongamia glabra* presents a transverse section resembling that of v. 21360 so far as the distribution of bands of parenchyma and the scattered arrangement of the vessels are concerned; *Pongamia*, however, has only 1- or 2-seriate rays, the vessels are more definitely related to the parenchyma bands than in v. 21360, and the rays and parenchyma, as seen in tangential section, are storied. *Baphia nitida* has also a general resemblance in transverse view to v. 21360, but its rays are mostly uniseriate, shallow, and, like the parenchyma, storied in arrangement. Many other Leguminous woods were examined, but throughout the whole series, in spite of superficial resemblances of transverse sections in certain cases, there was no one type which proved to be really similar to v. 21360 when a closer examination of detail was made.

In the case of *Cola nitida* (Sterculiaceae) a transverse section was found to resemble that of v. 21360, not only in general type, but in many of its details also: for example, in the relative extent of the fibre and parenchyma zones, the comparatively narrow rays, the arrangement and distribution of the vessels, and their variable degree of enclosure in the parenchyma (cf. 1, pp. 358, 360; Pl. XII, Fig. 5). In longitudinal section, also, the vessels of *C. nitida* are like those of v. 21360 in being rather short and completely perforate; while the rays of both types are variable in depth, the uniseriate being much less deep than the multiseriate examples. The multiseriate rays of *C. nitida* are, it is true, a little broader and considerably deeper than those of v. 21360; but in a type where there is so much variation in the vertical extent of the rays, this would not seem to constitute a serious difference. In most respects, therefore, *C. nitida* and v. 21360 are very similar in structural type; the general texture and the size of the elements in the two specimens under comparison are also similar.<sup>1</sup> The parenchyma of *C. nitida*, however, is storied (1, p. 360; Pl. XII, Fig. 7), while it is non-storied in v. 21360; and this is sufficient to constitute a fundamental difference between the two woods (1, p. 360).

Certain other members of the Sterculiaceae showing the *Ficus*-structure—*Sterculia elegantifolia*, for example—have also the storied arrangement of parenchyma, and cannot, therefore, be compared with v. 21360.

Various forms scattered throughout the Dicotyledons—for example, *Lophira procera* (Ochnaceae) and *Rinorea cibbiensis* (Violaceae)—show resemblances in general type to v. 21360, that is, in the possession of

<sup>1</sup> See, however, 1, p. 361, concerning the use of texture and size of elements in comparisons of timber specimens.

alternating bands of fibres and parenchyma; all such types were, however, rejected in comparison with the East African fossil, owing to differences in detail of structure.

While the *Ficus*-structure is frequently combined with the storied arrangement of parenchyma amongst recent timbers, this is not invariably the case. Various members of the family Guttiferae are like the fossil v. 21360 in possessing alternating bands of hard and soft tissue and *non-storied* parenchyma. In the material of *Calophyllum amonium*, *Garcinia* spp., and *Mesua ferrea* examined, the bands of parenchyma were very narrow; the relative proportions of fibre and parenchyma were found to be more similar to those of v. 21360 in *Rheedia* and *Symphonia*. In *Rheedia*, however, the vessels are enclosed in the parenchyma bands; and it is in *Symphonia* that the nearest approach to the structure of v. 21360 was discovered. The structure of *S. globulifera* resembles that of v. 21360 in that the bands of parenchyma and fibres are of a similar relative width; the vessels are scattered, occurring singly or in pairs, or occasionally in small groups or radial rows of from 3 to 5, while they are only partially enclosed in the parenchyma bands: in some cases tyloses are present; the rays, as in the fossil, are not very strongly defined in transverse section, and they are of similar extent and type, the size of the cells being somewhat variable, though the upper and lower marginal cells are less markedly different from the others in size and shape than in v. 21360: occasionally a ray is accompanied in the fibre bands by parenchyma cells, though these do not, in the material examined, form continuous radial rows, such as occur, in a few instances, in v. 21360 (cf. 1, pp. 358, 360, and Pl. XII, Figs. 6 and 8, with the description of the fossil, pp. 746 and 747 and Text-fig. 1a and b, of the present paper).

In the available material of *S. globulifera* the texture is considerably coarser than in v. 21360, the elements being larger. Photographs of other species of *Symphonia* given by Lecomte—for example, *S. fasciculata* (20, Pl. XXXVIII; see also Pl. XXXVI)—indicate, however, that there are variations within the genus in this respect, though it must be borne in mind that differences in the size of the elements cannot be used in comparison unless material from comparable regions of plants is under consideration.<sup>1</sup>

This being the case, *type of structure* is all that may validly be used in these comparisons; and the type of structure shown by v. 21360 is nearest to that shown, amongst recent timbers examined, by species of *Symphonia*, a genus ranging from Africa to tropical America, and having its centre of distribution in Madagascar.

<sup>1</sup> See 1, pp. 361, 362. It is for this reason that, in these comparisons, no reference is made to the *actual* size of the elements; although, for purely descriptive purposes, measurements are generally given in the accounts of the fossil material.

As might be expected from the present wide distribution of the *Ficus*-structure, this type is also known in a variety of fossil woods. The comparisons of these fossils with recent woods, and the reference of many of them to families and groups are unconvincing and unsatisfactory; a review of them has, however, been made, in order to determine whether any wood, similar in structural detail to v. 21360, and co-existent with it, has been described. Amongst fossil woods showing the *Ficus*-structure may be mentioned:

*Ficoxylon*, noted by Schenk (28, p. 899) as possessing numerous tangential bands of parenchyma. This 'genus' is represented by *F. cretaceum*, a Tertiary wood of Egypt and Tunisia, described and figured by Schenk (27, p. 14; Pl. V, Figs. 17-19), and compared with the wood of *Ficus Sycomorus*; and by *F. tropicum* (= *Ungerites tropicus*), from the Tertiary of Bohemia, described by Schleiden (29, p. 37), and compared by him with those members of the Leguminosae showing a similar arrangement of parenchyma; Felix, later, was of the opinion that this wood is close to that of *Ficus cordata* (9, p. 81).

*Taenioxylon* (Felix, 8, p. 10). In this 'genus', characterized, as its name implies, by a 'banded' structure, the actual *Ficus*-type, with *continuous* bands, seems only to occur where the vessels are numerous; this indicates that the parenchyma is definitely in connexion with the vessels. Various 'species' of *Taenioxylon* are figured by Felix, in his account of West Indian fossil woods, as showing structures of the general 'Leguminous' type, with irregular patches of parenchyma, sometimes elongated, surrounding the vessels (8, Pl. I, Figs. 1-11); these patches naturally unite into bands where the vessels are closely set (see *T. pannonicum*, 12, p. 145; p. 27, Figs. 1 and 2).

*Taenioxylon* ranges in horizon and area from the Upper Cretaceous of Brunswick (*T. varians*; Felix, 8, p. 10; Pl. I, Figs. 3 and 4), to the Pliocene (?) of the Philippines (*T. eperuoides*; Felix, 11, p. 491; Pl. XII, Figs. 5 and 6); and, while some of its 'species'—for example, *T. eperuoides*, *T. ingaeforme*, and *T. multiradiatum*—are compared with various Leguminous types, others are admittedly *incertae sedis*.

*Nicolia*. The 'species' of this undoubtedly highly composite 'genus' range from the Upper Cretaceous to the Pliocene strata of Northern Africa and elsewhere (cf. Edwards, 7, pp. 55-7). Of these species, *N. aegyptiaca* (see Unger, 34, p. 523) is described as a wood possessing from 1- to 3-seriate rays, and large vessels arranged singly or in pairs. Schuster's figure (30, Pl. II, Fig. 10) indicates a wood of the *Ficus*-type, with parenchyma in tangential bands, though previous figures of '*N. aegyptiaca*' do not show this structure (see Unger, 36, Pl. I, Fig. 1; and Schenk, 27, Pl. III, Fig. 7); Schenk, however, comments on a certain agreement between *Nicolia* and *Ficoxylon cretaceum* (27, p. 14). Unger (37, p. 214) compares *N. aegyptiaca*

with the Sterculiaceae, and his Pl. I, Fig. 2, shows indications of storying of the rays, as in that family. According to Schuster, *N. aegyptiaca* (including *N. Wiedmanni* (Hofmann, 16)) should be referred to the Sterculiaceae, although his Fig. 11 (30, Pl. II) does not show storied parenchyma in tangential section of the wood. On the other hand, *Nicolia Oweni* (Carruthers, 6, p. 310, Pl. XIV, Figs. 1 and 2), with which may be united Hofmann's *N. minor*, is suggested by Schuster to have affinities with the Caesalpinioideae amongst the Leguminosae, rather than with the Sterculiaceae.

Without more evidence than is available in the case of these fossils, their suggested affinities must, of course, be left an open question. Comparisons, however, established between such forms as, for example, *Taenioxylon eperuoides* and *T. ingaeforme* and certain members of the Leguminosae; between *Nicolia aegyptiaca* and the Sterculiaceae; and between *Ficoxylon* and *Ficus*, do not, in the first place, suggest similarity of these fossils to v. 21360 in detail of structure; and certainly, none of the descriptions and figures examined by the writer has revealed any close resemblance between the East African fossil and other Tertiary or Upper Cretaceous forms possessing the *Ficus*-structure.

This being the case, the recent genus *Symphonia* stands, amongst the recent and fossil forms reviewed, as displaying the type of structure nearest to that of v. 21360, for which, in accordance with the views expressed in the introductory paper of this series (1, p. 363), the name *Dryoxylon symphonioides* is therefore proposed.

### 3. *Diagnosis.*

Specimen v. 21360, Nat. Hist. Mus. Coll.

*Dryoxylon symphonioides* sp. nov.

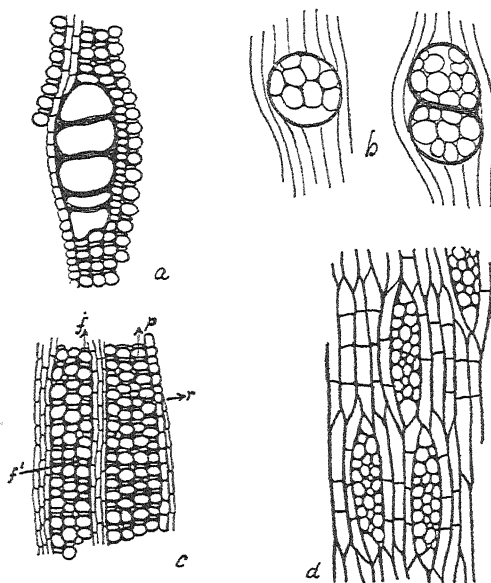
A dicotyledonous wood of the '*Ficus*-type', i.e., possessing concentric and alternating bands of fibres and parenchyma; seasonal zoning not marked; vessels completely perforate, generally single or in pairs, scattered, typically only partially enclosed in the parenchyma bands, in some cases showing evidences of tyloses; rays fairly numerous, not strikingly distinct in transverse section, from 1 to 4 cells wide, slightly heterogeneous, and the wide rays much deeper than the uniseriate rays; rays and parenchyma not storied as seen in tangential section; the wood as a whole showing resemblances to that of various species of the recent genus *Symphonia*.

Specimen v. 21361 (sections *a*, *b*, and *c*).

1. *Description.* (Text-figs. 2, *a*, *b*, *c*, and *d*; Pl. XXIX, Figs. 2, 3, and 4).

Specimen v. 21361 is an imperfectly preserved isoporous wood, with only very slight indications of seasonal zoning, marked in transverse

section by the narrowing of the small elements of the wood. The vessels are large, an average size being  $290\ \mu$  (radial diameter) by  $270\ \mu$  (tangential diameter); they are not very numerous and are distributed fairly evenly.



TEXT-FIG. 2. v. 21361. *a.* Transverse section of a radial group of vessels. *b.* Transverse sections of vessels with tyloses. *c.* A portion of a transverse section showing rays and the alternating arrangement of fibres (*f.*) and parenchyma (*p.*). At *r*, a biseriate ray becomes uniseriate in passing outwards towards the periphery of the stem. Very small fibres are occasionally shown between the larger cells, as at *f.* *d.* A portion of a tangential section showing rays and storied parenchyma. (The magnification in each case is 60 diameters.)

They typically occur singly (Pl. XXIX, Figs. 2 and 3), only occasionally in pairs, and rarely, in the later-formed wood, in radial rows of from 3 to 5 or more (Text-fig. 2 *a*); this latter arrangement, however, seems to be frequent in the regions immediately surrounding the pith, so far as preservation allows of determination. Here and there, in both transverse and longitudinal sections, are indications of narrow xylem parenchyma cells surrounding the large vessels, but usually the tissues are indistinct here; in any case, it would seem that surrounding xylem parenchyma is very small in amount, comprising only 1 or 2 cell-layers. In one place, the vessels appear to contain tyloses (Text-fig. 2 *b*). The actual structure of the vessels is not easy to determine owing to imperfect preservation, but they are apparently rather short and completely perforate, while there are numerous small, closely-set pits on their walls.

Very characteristic is the regular radial arrangement of the small elements of the wood as seen in transverse section. They are of three kinds: (1) rounded elements having an average diameter of  $25\ \mu$  (Text-fig.

2 *c*, *p*); (2) smaller elements, typically elliptical in section, measuring from 20 to 25  $\mu$  tangentially, by 10 to 12  $\mu$  radially (Text-fig. 2 *c*, *f*); these two types of cell occur alternately in each radial series, as shown in Pl. XXIX, Figs. 2 and 3, and also in Text-figs. 2 *a* and *c*; (3) still smaller elements, which may occasionally be seen to occur singly or in groups of 2 or 3, in the interstices between the radially-arranged cells (Pl. XXIX, Fig. 3, *f'*, and Text-fig. 2 *c*, *f'*). The preservation of the fossil is not sufficiently good for these very small cells to be generally detected; nor can the thickness of any of the cell walls be determined with any degree of accuracy. In longitudinal section, the two types of smaller element, *f* and *f'*, are seen to be narrow, elongated, and pointed, and are therefore of the nature of fibres; while the wider elements are parenchyma, being short, and, for the most part, square-ended; inclined end walls, however, occur at the levels of the upper and lower margins of the rays, as seen in tangential section, giving a tier-like or storied effect to the parenchyma (Pl. XXIX, Fig. 4; Text-fig. 2 *d*). In neither fibres nor parenchyma can pitting of the walls be observed; in the case of the fibres there are occasional traces of what may be septation, but the condition of the sections is not sufficiently good to allow of certainty upon this point.

The rays have a general storied arrangement as seen in tangential section (Pl. XXIX, Fig. 4); they are very numerous and closely set, being separated by from 2 to 6 rows of small elements (Pl. XXIX, Fig. 2; Text-fig. 2 *c*). They are spindle-shaped, from 10 to 12 cells in height, uniseriate at the upper and lower margins, and from 2- to 4- (very typically 3-) seriate in the wider parts (Pl. XXIX, Fig. 4; Text-fig. 2 *d*). The rays are heterogenous, the cells of their upper and lower margins being larger than the others, while they are also elongated instead of rounded, as seen in tangential section (Text-fig. 2 *d*); there is, moreover, variation in the size of the rounded cells (Text-fig. 2 *d*).

Evidence of secretory sacs in the wood is uncertain, as the tissues are much broken up, and many spaces occur which may or may not have been originally of that nature.

The inner margins of the wood and the pith are also very disintegrated; the pith is, however, apparently composed of small-celled parenchyma.

## 2. *Comparison with recent and other fossil types.*

Amongst recent timbers examined, the type of structure exhibited by v. 21361 was found only amongst the Malvales. *Premna velutina* (Verbenaceae), it is true, shows a certain resemblance to the fossil in transverse section, but in tangential section, its rays and parenchyma are not storied, a fact which excludes it at once from comparison with v. 21361.

Of the Malvaceae, *Hibiscus lasiococcus*, as figured by Lecomte (20,

p. 31), shows similarity in transverse section to v. 21361; in tangential section, however, not only are the rays much more variable in depth and width, but the cells composing them display a greater range of size, than is the case in the fossil type. Weale's photograph of the transverse section of *Hibiscus heterophyllus* (38) also shows the same general appearance to that of v. 21361, except that the vessels are more grouped than is typical of the fossil, at any rate in the later-formed wood.

Amongst the Sterculiaceae, there is a considerable range of structural type, various members of the family showing the *Ficus*-structure, as already noted; others (for example, *Triplochiton* spp.) approach the structure of v. 21361. While, however, *Triplochiton* shows the radial arrangement of larger and smaller elements with a packing of still smaller elements in the interstices, and the large solitary vessels and storied parenchyma possessed by v. 21361, the rays are much deeper than in the fossil, and much more heterogeneous.

The members of the Bombacaceae, particularly those belonging to *Bombax* itself, show a very close resemblance to v. 21361, though in no case examined, was there exact similarity. In *Bombax malabaricum*, the rays are much broader and deeper than in the fossil; and in *B. insigne*, while the rays are more similar in width, they are again deeper; moreover, the alternation of larger and smaller cells is apparently not so regular in this species as it is in v. 21361. In a specimen of *B. ceiba* was found the nearest approach to the structure of the fossil, for its rays are more shallow and their constituent cells less variable in size than in the other species of *Bombax* examined; the main difference between this specimen of *B. ceiba* and v. 21361 consists in the fact that the rays of the recent type are less uniform in depth than in the fossil, so that the storied effect as seen in tangential section is not so evident. A minor difference is that the rays of *B. ceiba* are on the whole slightly narrower than in v. 21361.

Various fossils have been referred to Malvacean, particularly Sterculiacean, affinities, for example, *Nicolia aegyptiaca*, *Sillimannia texana*, *Dombeyoxylon* spp., *Staubia eriodendroides*, and *Tarrietioxylon sumatrense*. Of these, *Nicolia aegyptiaca*, as noted on p. 751, apparently shows the *Ficus*-structure, and may therefore be rejected in comparison with v. 21361; while information concerning *Sillimannia* is too insufficient to be of use (see Unger, 34 and 35).

*Dombeyoxylon aegyptiacum* from the Oligocene (?) of Egypt, was compared by Schenk (27, p. 13), especially with the wood of *Ruizia* and *Guazuma* amongst recent Sterculiaceae; by Felix (13, p. 522) with *Guazuma*; and by Schuster (30, p. 12; Pl. III, Fig. 18) with *Eriodendron* (Bombacaceae). *D. affine*, from the Tertiary of Abyssinia, was described and figured by Felix (13, p. 520; Pl. XXV, Figs. 2, 3, and 5), and compared by him with the recent species *Dombeya mollis* (Sterculiaceae); while *D. jacksonensis*, from

the Eocene of Louisiana, was compared by Berry with *D. affine* (3, p. 181; Pls. XXXVI and XXXVII).

Of these, only *D. aegyptiacum* shows a type of structure comparable with that of v. 21361, although the rays appear to be closer and narrower than in the East African fossil; no tangential section of *D. aegyptiacum* is, however, figured, so that it is not possible to compare it with v. 21361 with regard to the depth, composition, and arrangement of its rays.

*Staubia* was stated by Felix (10, p. 28; Pl. II, Figs. 2, 4, 5, 6, and 8) to be close to *Dombeyoxylon*, and *Staubia eriodendroides*, from the Tertiary of Hungary, to be intermediate between *Pterospermum* (Sterculiaceae) and *Eriodendron* (Bombacaceae). This type shows the tiered structure in tangential section, but the rays are very heterogeneous, much more so than in v. 21361.

*Tarrietioxylon sumatrense*, from the Tertiary of South Sumatra, compared with *Tarrietia*, and referred to the Sterculiaceae by Kräusel (19, p. 259; Pl. II, Fig. 4; Pl. IV, Figs. 2, 3, and 6; Pl. VI, Figs. 4, 5, and 9; Text-fig. 23), also shows tiered structure; it possesses, however, more closely set vessels than v. 21361, and there are also crystal-cells.

From available information concerning these fossils, it does not appear that any one of them shows close resemblance to v. 21361; this East African type seems to present the greatest amount of similarity to the wood of the recent large and widely distributed tropical genus *Bombax*, especially to that of *B. ceiba*, amongst the species examined; the name *Dryoxylon bombacoides* is therefore proposed as being descriptive of its structural type.

### 3. *Diagnosis.*

Specimen v. 21361, Nat. Hist. Mus. Coll.

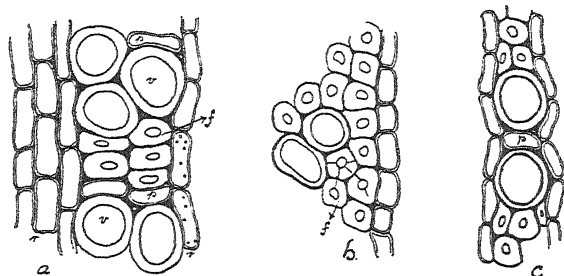
*Dryoxylon bombacoides* sp. nov.

A dicotyledonous wood, with only slight indications of seasonal zoning, characterized by a regular, radial arrangement of alternately placed fibres and parenchyma cells, the fibres being rather smaller in section than the parenchyma; the resulting interstices between the rows of cells occupied (so far as preservation of the wood allows of determination) by very small fibres placed singly or in small groups; vessels apparently completely perforate, large, not numerous, evenly distributed, typically isolated in the later-formed wood, frequently grouped in the earlier wood; rays numerous and closely set, typically 3-seriate, and from 10 to 12 cells in height, heterogeneous; rays and parenchyma storied as seen in tangential section; the wood as a whole showing resemblances to that of the recent genus *Bombax*.



Specimen v. 21362 (sections *a* and *c*)1. *Description.* (Text-figs. 3, *a*, *b*, and *c*; Pl. XXIX, Figs. 5, 6, and 7).

Specimen v. 21362 is a portion of a small dicotyledonous stem, about 3 cm. in diameter, bearing the base of a lateral branch, the



TEXT-FIG. 3. v. 21362. *a*. Transverse section of a portion of the secondary tissues, showing the vessels (*v*), fibres (*f*), parenchyma (*p*), and two rays (*r*). Two cells of the uniseriate ray are pitted. *b*. Transverse section of a portion of the secondary tissues showing the pitting of the fibres (*f*). *c*. Transverse section of a portion of the secondary tissues, showing a parenchyma cell (*p*) between two uniseriate rays. (The magnification in each case is 250 diameters.)

width of the specimen through the branch-base being 3.8 cm.; the outer tissues are not represented. Preservation is not good except in the centre of the main stem. The pith has a close, regular, compact texture, the cells being rounded or hexagonal in transverse section, and having fairly thick walls (Pl. XXIX, Figs. 5 and 6). They vary in diameter from  $18\mu$  to  $49\mu$ , the smaller cells being situated at the periphery of the pith, though there is no definite cell-layer separating the primary wood from the pith. None of the cells appears to be secretory or crystal-containing.

The primary wood forms an almost unbroken undulated ring round the pith, having no distinct bundles, though here and there are indications of grouping of the elements (Pl. XXIX, Figs. 5 and 6). This first-formed wood consists of fairly regular radial rows of small elements, rounded as seen in transverse section, and varying in diameter between  $18\mu$  and  $27\mu$ ; their walls are only slightly thickened. The rows consist of from 5 to 9 of these radially-placed elements, and between the rows are what appear to be fibres and certain thinner-walled cells, probably parenchyma. Here and there, multiseriate and uniseriate rays may be traced from the pith between the rows of rounded elements, and for some distance through the subsequently-formed wood (Pl. XXIX, Fig. 6).

The secondary wood consists of vessels, thick-walled fibres, a small amount of xylem parenchyma, and numerous narrow rays (Pl. XXIX, Fig. 5). Seasonal zoning is recognizable, though not strongly marked by any definite structural feature (Pl. XXIX, Fig. 5); there is, however, a very

slight narrowing of the elements at the end of each ring of growth, and a tendency to the regular arrangement of the vessels at its commencement. The width of the rings is rather variable, and not easy to determine on account of the frequent lack of clear definition; they are, on the whole, very narrow, especially in the later-formed wood.

The wood is practically isoporous, and the vessels are numerous and fairly evenly distributed (Pl. XXIX, Fig. 5), though here and there they occur more closely at the beginning of a seasonal zone. The vessels are approximately circular in transverse section (Pl. XXIX, Figs. 5 and 6; Text-figs. 3 *a* and *c*), and their openings vary from  $18\ \mu$  to  $36\ \mu$  in diameter, the most frequent measurements being between  $27\ \mu$  and  $32\ \mu$ . Their walls are comparatively thick (Text-figs. 3 *a*, *b*, and *c*), and they are usually isolated, but sometimes occur in pairs, or in radial rows of three or more. Longitudinal sections of the wood are too imperfect to show the pitting of the vessel walls, or to give any clear indication whether the perforations are scalariform or complete.

The fibres of the wood are somewhat square to hexagonal in transverse section (Text-figs. 3 *a*, *b*, and *c*), varying in diameter from  $14\ \mu$  to  $23\ \mu$ . They are very thick-walled; here and there, pitting may be detected (Text-fig. 3 *b*), and in one or two cases, the pits appear to be bordered. The course and extent of the fibres are not distinct in the longitudinal sections of the specimen.

Here and there between the vessels, or partially surrounding them, or stretching between two closely-set rays, are comparatively thin-walled parenchyma cells (Text-fig. 3 *c*). These cells may be rounded, but are usually elliptical in shape, the tangential diameter being the greater; they are rather variable in size, measurements ranging from  $9\ \mu$  tangentially by  $6\ \mu$  radially, to  $18\ \mu$  tangentially by  $9\ \mu$  radially. Pitting cannot be distinguished in either transverse or longitudinal sections.

The rays are numerous and closely-set, and are from 1 to 4 cells wide, as seen in transverse section; narrowing to one cell row, and complete dying out may occasionally be observed in the case of 3- or 4-seriate examples; this appearance may be due to the oblique course of the rays. The wider rays appear to be, in some cases at least, markedly heterogeneous, for they possess, as seen in tangential section, uniseriate margins consisting of vertically elongated parenchyma cells (Pl. XXIX, Fig. 7); there are as many as 4 or 5 of these cells in a vertical series, giving a general effect of the rays merging into vertical rows of parenchyma cells. The cells of the rays are, on the whole, small, though variable in size, and, as seen in transverse section, they are more isodiametric in the case of the uniseriate rays, and brick-shaped in the 2- to 4-seriate rays. Measurements of the isodiametric cells indicate an average diameter of  $19\ \mu$ ; while the brick-shaped cells vary between  $9\ \mu$  and  $14\ \mu$  tangentially, and between  $18\ \mu$  and  $27\ \mu$  radially; the

vertically elongated marginal cells, as seen in tangential section of the wood, measure about  $30\mu$  (vertically) by  $10\mu$  (horizontally). The rays are from 15 to 25 cells deep; there is no trace of storied arrangement. Usually no pitting of the ray-cells may be seen, but in a few cases their end walls appear to be pitted, and occasionally the horizontal walls definitely show minute pits (Text-fig. 3 a).

## 2. Comparison with recent and other fossil types.

Specimen v. 21362 is an example of the 'generalized type' of wood-structure described and figured in the introductory article of this series (1, p. 357; Tables I and II; Pl. XII, Figs. 1-4). So far as its general characters, as seen in transverse section, are concerned—the fine, close texture; small scattered vessels; thick-walled fibres, with, at any rate occasional, bordered pits; diffuse and scanty xylem parenchyma; and many narrow rays—the forms with which it may be compared are numerous, and widely distributed both in space and time.

The longitudinal sections of this fossil are unfortunately not good, and various details of structure are indeterminate. This is the case with regard to the perforations of the vessels; it is impossible to say whether they are simple or scalariform, or whether, as in most of the forms included in Table I (1, p. 355), both types of perforation occur. The tangential section, however, shows very plainly, here and there, that the rays are heterogeneous, the margins of some of the wider examples being uniseriate, and composed of vertically elongated cells; this structural feature provides a basis for a certain amount of discussion concerning the similarity of v. 21362 to other forms.

In *Aptiana radiata*, Dr. Stopes has figured rays with elongated marginal cells; these cells are, however, longer than in v. 21362, and there are fewer of them—only 1 or 2—in a vertical series (33, Text-fig. 92). Dr. Stopes remarks that she has observed rays with these vertically elongated marginal cells in only a limited number of recent species, outstanding examples being *Cliftonia ligustrina* (Cyrillaceae), *Viburnum rufotomentosum* (Caprifoliaceae), and *Ilex decidua* (Aquifoliaceae) (33, p. 292). According to the writer's experience at the moment, however, such rays appear to present an extreme degree of heterogeneity in the elongation of their marginal cells; a lesser degree is exhibited by the rays of such a type as *Curtisia faginea*, where the uniseriate margins of the 2- and 3-seriate rays, though very deep, are composed of cells much less vertically elongated than in *Aptiana* and the recent species observed by Dr. Stopes; the depth of the ray-margin in this case is due, not to the length of the individual cells, but to the number of cells in the series, six, or even more, occurring in *Curtisia*. In *Viburnum opulus*, the biseriate rays often show deep uniseriate margins composed of cells of varying vertical extent, some of them being only slightly elongated; while in *Myrica*

*rubra*, biseriate rays may possess uniseriate margins from one to several cells deep, the cells being on the whole less elongated than in *V. opulus*; in some cases, in fact, they are practically isodiametric as seen in tangential section. It seems, therefore, impossible to consider the rays of *Aptiana*, *Cliftonia ligustrina* and the other species mentioned by Dr. Stopes, with their very elongated marginal cells, as being in a distinct class, for there are all degrees of marginal-cell elongation connecting the *Aptiana* type with the *M. rubra* type, which, in its turn, marks a transition to the homogeneous type of ray; specimen v. 21362, apparently shows an intermediate stage, possessing a *degree* of heterogeneity somewhat comparable with that of *C. faginea*, though the ray margins are possibly less deep on the whole than in the recent species; so few rays were distinguishable in v. 21362, however, that it would be unwise to establish any very definite comparison between it and other types in this respect.

The primary wood is well represented in v. 21362; unfortunately, however, primary wood of most of the types used in comparison with the fossil was not available; and of the few cases examined, there was not one which showed a structure exactly comparable with that of v. 21362. An unnamed species of *Lonicera* approached the primary wood-structure of the fossil in the occasional regular radial arrangement of small rounded elements; but there were fewer cells in a radial row, as seen in transverse section, and the ring was rather more broken. In *Aptiana*, on the other hand, though the ring of primary elements is almost continuous as in v. 21362, it appears to be wider, for Dr. Stopes indicates that it consists of from 12 to 15 cell rows, as against from 5 to 9 rows in v. 21362.

While, therefore, a large number of forms, both recent and fossil, was discovered which agree with v. 21362 in general characters, no one of them was found to conform to it in all details of structure, and it is impossible to compare it with one type more than with any other. Under these circumstances, it is considered advisable to identify the fossil with its place of origin rather than to compare it with any known type; the name *Dryoxylon kenyense* is accordingly suggested.

### 3. *Diagnosis.*

Specimen v. 21362, Nat. Hist. Mus. Coll.

*Dryoxylon kenyense*, sp. nov.

A dicotyledonous wood, showing a generalized type of structure and a close, fine texture common to a large number of recent and fossil forms; vessels small, isolated or in pairs, or, occasionally, in short radial rows; fibres thick-walled and pitted; parenchyma diffuse and scanty; rays numerous and narrow, from 1- to 4-seriate, heterogeneous, occasionally, in the case of the wider examples, possessing deep uniseriate margins.

Seasonal zoning recognizable, though not strongly marked, by a slight narrowing of the elements at the end of each growth-ring, and a tendency to the formation of a pore-zone at its commencement.

Specimen v. 21363 (section *a*).

1. *Description.* (Text-fig. 4, *a*, *b*, and *c*; Pl. XXIX, Fig. 8).

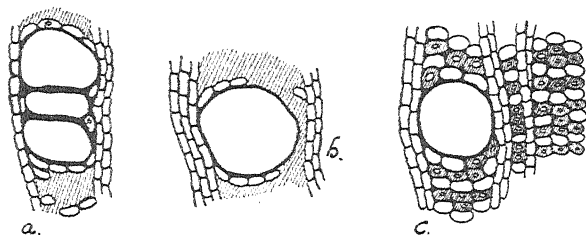
Specimen v. 21363 is a portion of the woody cylinder of a dicotyledonous stem. The pith and primary wood, and also the outer tissues of the stem are entirely lacking, so that no idea is given of its original dimensions. The wood is very imperfectly preserved, and, moreover, at one side of the stem, disturbance of the regular course of the elements is caused, apparently, by the base of a small branch; so that it is only from parts of the transverse section that an indication of the type and structure of the wood may be obtained. A characteristic pattern is given to the wood by numerous tangential and uniseriate rows of parenchyma cells (Pl. XXIX, Fig. 8). Seasonal zoning may be recognized with the naked eye, but owing to the poor state of preservation of the specimen and blurring of the cell outlines, it is not easy to determine structural difference of the elements microscopically; there are indications, however, that the zoning may be due to the absence of the tangential lines of parenchyma, which are elsewhere very closely set.

The wood is isoporous; the vessels are numerous and occur singly (Text-figs. 4 *b* and *c*), or in pairs, or sometimes in radial rows of three (Pl. XXIX, Fig. 8; Text-fig. 4 *a*), or more. They are rounded to elliptical in transverse section, the tangential diameter tending slightly to exceed the radial, especially where the vessels form a series of two or more. Their most frequent size is  $90\mu$  (tangentially) by  $81\mu$  (radially). The longitudinal sections give no idea of the length of the vessels, or of the nature of their perforations; their walls are not greatly thickened, and only in one case is a close network of small pits indicated.

The fibres of the wood are usually very blurred; in a few cases, however, the cell outline and lumen may be detected. The walls are thick, and no pitting is visible. The external measurements of the cells are similar to those of the parenchyma cells, the tangential diameter being slightly the greater.

Fairly thin-walled parenchyma cells occur in tangential uniseriate lines of varying regularity. These lines are closely set, being separated very generally by a single series of fibres (Text-fig. 4 *c*). Parenchyma frequently occurs in connexion with the vessels, partially surrounding them (Text-figs. 4 *a* and *b*). The cells are rounded to elliptical in shape, as seen in transverse section (Pl. XXIX, Fig. 8; Text-fig. 4 *c*); in the latter case, the tangential is the greater dimension. The most frequent size is  $18\mu$  (tangentially) by  $14\mu$  (radially). No pitting can be distinguished.

The rays are from 1 to 4 cells wide as seen in transverse section; 2- and 3-seriate rays appear to be the most frequent (Pl. XXIX, Fig. 8; Text-figs. *a*, *b*, and *c*). Their radial course is often deflected by the vessels



TEXT-FIG 4. v. 21363. *a*. Portion of a transverse section, showing a radial row of vessels between two rays. Thin-walled parenchyma cells (*p*.) are in contact with the vessels; the shaded areas represent the indistinct fibres. *b*. A large vessel in transverse section, partly surrounded by parenchyma cells. *c*. A portion of a transverse section, showing the arrangement of thick-walled fibres and parenchyma. (The magnification in each case is 125 diameters.)

(Pl. XXIX, Fig. 8). They are numerous and closely arranged, with from six to four, or fewer, radial rows of elements between each two; it is not possible to determine their vertical extent or their arrangement from the longitudinal sections. In the case of the uniseriate rays, the cells are almost isodiametric, while in the wider rays, the cells are elongated radially (Pl. XXIX, Fig. 8, *r*); this may indicate that the rays are slightly heterogeneous. The cell-outlines are blurred and measurement is therefore difficult; approximate measurements are, however, in the case of a uniseriate ray,  $18\mu$  (tangentially) by  $20\mu$  (radially), and in the wider rays,  $18\mu$  (tangentially) by  $23\mu$  (radially). No pitting can be distinguished.

## 2. Comparisons with recent and other fossil types.

The outstanding feature of v. 21363 consists in its numerous uniseriate tangential lines of parenchyma cells; an examination of recent types indicates that a similar structure is of frequent occurrence in fairly diverse groups of woody Dicotyledons.

The Anonaceae, as a family, are characterized by the possession of tangential lines of parenchyma as seen in transverse section of the wood; these lines are occasionally uniseriate, as in *Miliusa velutina*, though not so consistently as in v. 21363; moreover, in no case do they appear to be as closely-set as in the fossil, and they are typically more regular in arrangement.

'Fine transverse bars or concentric lines' of parenchyma are also a feature of the Sapotaceae and Ebenaceae (Gamble, 15, pp. 443 and 452); but in these forms again, the parenchyma bands are often biseriate, and are typically more widely separated than in v. 21363, though certain species of *Diospyros* approach the fossil in this respect; the predominantly uniseriate

rays of the recent genus, however, would seem to differentiate it from v. 21363. An unnamed species of *Royena* (Ebenaceae) showed parenchyma lines frequently separated by only one series of fibres, though they were much less regular than in v. 21363, and the numerous vessels much less frequently grouped; also, the rays of *Royena* sp. were, on the whole, broader and less closely arranged.

*Hopea odorata* (Dipterocarpaceae) shows a similar general type of structure in transverse section, but different sections indicated considerable irregularity in the disposition of the parenchyma lines, and it was impossible to compare any one of them definitely with v. 21363.

Amongst the Lecythidaceae, *Lecythis* spp., and *Foetidia mauritiana* possess more or less regular tangential lines of parenchyma; these are, however, further apart than in v. 21363, and, in the material of these species available for examination, the rays were uni- or biseriate. In this family also, *Barringtonia acutangula* shows the same general type of structure; it seems, however, to have more irregular parenchyma lines, vessels more grouped, and more ray tissue than the fossil—differences which are emphasized in the related species, *B. racemosa*.

The Geraniales include a number of types possessing fine tangential lines of parenchyma. A tentative comparison was originally made between v. 21363 and *Humiria floribunda* (Humiriaceae); a wider acquaintance with the recent species, however, has confirmed the impression that its parenchyma lines are less regular and distinct than those of v. 21363, the rays finer, and the vessels less numerous.

Various members of the Rutaceae, for example, *Calodendron capensis*, also show uniseriate tangential lines of parenchyma, but these are more widely separated than in v. 21363.

The woods of the Euphorbiaceae exhibit a very considerable variety of structural type (cf. 26, p. 369), there being many cases where the parenchyma occurs in fine, often closely spaced, tangential or concentric lines, as, for example, in *Croton oblongifolium*, *Cyclostemon microphyllus*, *Drypetes* spp., *Aporosa* spp., and others.

It must be remembered that, in the case of v. 21363, the longitudinal sections do not yield sufficient data for comparison; reference can therefore be made only to transverse sections of recent timbers, and all comparisons are necessarily incomplete, being based upon these alone. Amongst the Euphorbiaceous species above mentioned, the nearest approach to the transverse section of v. 21363 was found in those of *Cyclostemon microphyllus* and *Drypetes* spp.; and so far as the spacing of the tangential lines of parenchyma is concerned, an unnamed species of *Drypetes* from Liberia showed the greatest similarity, for the lines were mostly one fibre series apart, as typically occurs in the fossil. The rays were perhaps rather more numerous than in v. 21363, and narrower, though 3-seriate examples

occurred here and there; a slight degree of heterogeneity of the rays was indicated in the transverse section of *Drypetes* sp., as in that of the fossil.

Various fossil types possessing tangential lines of parenchyma cells have been referred to the 'genus' *Ebenoxylon*, for example, *E. tenax*, from the Oligocene of Saxony, described and figured by Beck (2, p. 348; Pl. VII, Figs. 7-9). This, and other 'species' of *Ebenoxylon*, may be rejected in comparison with v. 21363, for the parenchyma is more widely separated and more irregular than in the East African fossil, *Jordania ebenoides* (= *Ebenoxylon ebenoides*; cf. Edwards, 7, p. 38), compared by Schenk with *Royena* and other forms, and provisionally referred to the Ebenaceae, has apparently uniseriate rays, and is therefore not comparable with v. 21363 (27, p. 10; Pl. IV, Figs. 13, 14; 28, p. 903).

*Sapotoxylon gumbelii*, from Tertiary (?) strata near Neuburg on the Danube, and *S. taeniatum* were described and figured by Felix (9, pp. 67 and 68; Pl. II, Figs. 5 and 8, and Pl. III, Figs. 5 and 6) as having narrow parenchyma lines, which are not, however, comparable with those of v. 21363, for they are widely separated as in the recent species of the Sapotaceae (and also Anonaceae) with which they are compared.

*Taenioxylon porosum*, from the Eocene of the Caucasus, may, according to Felix, also be a *Sapotoxylon* (14, p. 103; Pl. X, Fig. 3); this type possesses tangential parenchyma lines, the disposition of which is, however, certainly more comparable with that in the Sapotaceae than with that in v. 21363.

No fossil wood having a structure similar to the type presented by v. 21363 is apparently so far known; in view, therefore, of the similarity of the transverse section of *Drypetes* sp., to that of v. 21363, the name *Dryoxylon drypeteoides* is suggested for the East African fossil.

### 3. Diagnosis.

Specimen v. 21363, Nat. Hist. Mus. Coll.

*Dryoxylon drypeteoides*, sp. nov.

A dicotyledonous wood, having only slight indications of seasonal zoning; characterized by the possession of fine, uniseriate, closely-set, tangential lines of parenchyma; the intervening fibres thick-walled; vessels all similar in size, numerous, occurring singly or in pairs, or in radial rows of three or more; rays numerous and closely-arranged, from 1- to 4-, typically 2- or 3-seriate, apparently slightly heterogeneous; the transverse section of the wood as a whole showing similarity to that of *Drypetes*.

### III. SUMMARY.

Four fossil woods from the Miocene beds of Kikongo, Kenya Colony, are described and figured. In the absence of any associated vegetative



and reproductive remains to indicate their affinities, the specimens are referred to Schleiden's form-genus *Dryoxylon*, and compared with various recent and fossil timbers. The following specific references are proposed :

1. *D. symphonoides*: a wood of the 'Ficus-type' (i.e., with alternating zones of fibres and parenchyma), showing considerable similarity of structure to that of *Symphonia* spp.

2. *D. bombacoides*: compared with various Malvacean woods, particularly with that of *Bombax ceiba*.

3. *D. kenyense*: a wood of generalized type, such as occurs in many living genera and families throughout the Dicotyledons (e.g., in *Myrica*, *Cornus*, *Rhododendron*, *Viburnum*, &c.), and which is also to be found amongst the earliest woods so far known (e.g., in the Cretaceous forms *Aptiana radiata* and *Cornoxylon myricaeforme*).

4. *D. drypeteoides*: compared especially with the wood of *Drypetes* sp.

The very grateful thanks of the writer are due to Dr. W. D. Lang, Keeper of the Geological Department of the British Museum, for permission to publish the foregoing descriptions of Dr. Oswald's East African fossils; to Mr. W. N. Edwards, also of the Geological Department, for much valuable help with regard to the literature on fossil dicotyledonous woods; to Dr. L. Chalk, for the use of the collection of recent timbers in the Imperial Forestry Institute, Oxford; to Dr. Burt Davy, of the Imperial Forestry Institute, for herbarium material of recent genera used in comparison with the fossils; to Professor W. Dawson, of the Cambridge School of Forestry; and to Mr. A. L. Clinkard for the micro-photographs used in illustration of this paper.

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#### POSTSCRIPT.

While this paper was awaiting publication a letter from Mr. E. J. Wayland appeared in 'Nature' (129, 24, 1932) noting that recent discoveries have made it possible that the Karungu deposits may be Plio-Pleistocene rather than Miocene.

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## EXPLANATION OF PLATE XXIX.

Illustrating Miss Bancroft's paper On Some Fossil Dicotyledonous Woods from the  
Miocene (?) Beds of East Africa.

(Microphotographs).

Fig. 1. *Dryoxylon symphonioides*, specimen v. 21360. Transverse section (slide v. 21360 a), to show the general type of the wood, with alternating zones of parenchyma (*p.*) and fibres (*f.*), and the scattered distribution of the vessels; the rays are indistinct.  $\times 30$ .

Figs. 2-4. *Dryoxylon bombacoides*, specimen v. 21361.

Fig. 2. Transverse section (slide v. 21361 a), showing the alternating arrangement of fibres (*f.*) and parenchyma (*p.*), the heterogeneous rays (*r.*), and a large vessel (*v.*).  $\times 50$ .

Fig. 3. A portion of the same transverse section to show the details of structure on a larger scale; *f'*, very small fibres; the other lettering as in Fig. 2.  $\times 105$ .

Fig. 4. Tangential section (slide v. 21361 c), showing the general storied arrangement of the rays (*r.*) and parenchyma (*p.*).  $\times 30$ .

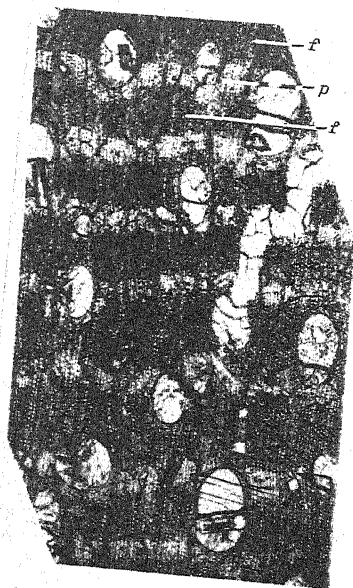
Figs. 5-7. *Dryoxylon kenyense*, specimen v. 21362.

Fig. 5. Transverse section (slide v. 21362 a), showing the pith (*m.*) and primary wood (*xy'*), and the secondary wood (*xy''*) with seasonal zoning; note the close arrangement of the narrow rays.  $\times 30$ .

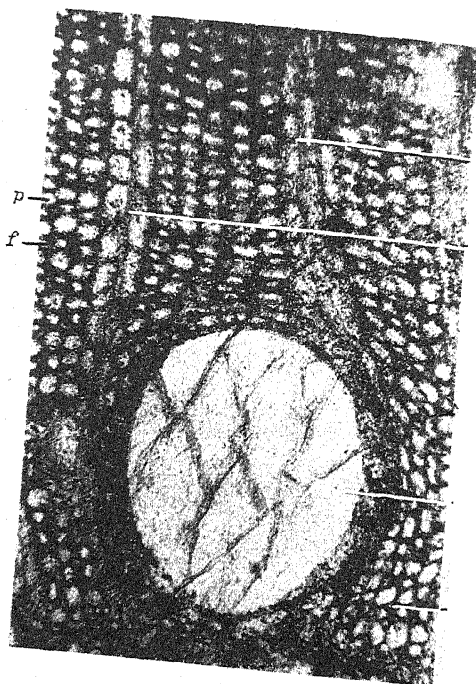
Fig. 6. A portion of the pith and of the primary and secondary wood from slide v. 21362 a, to show the narrow rays (*r.*) running from the primary to the secondary wood.  $\times 105$ .

Fig. 7. Tangential section (slide v. 21362 c) to show the heterogeneous rays.  $\times 200$ .

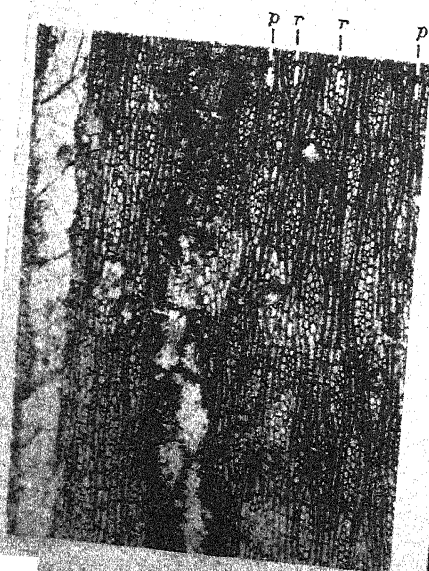
Fig. 8. *Dryoxylon drypeteoides*, specimen v. 21363. Transverse section (slide v. 21363 a), showing the numerous tangential and uniseriate lines of parenchyma (*p.*), the intervening fibres (*f.*), vessels (*v.*), and heterogeneous rays (*r.*).  $\times 105$ .



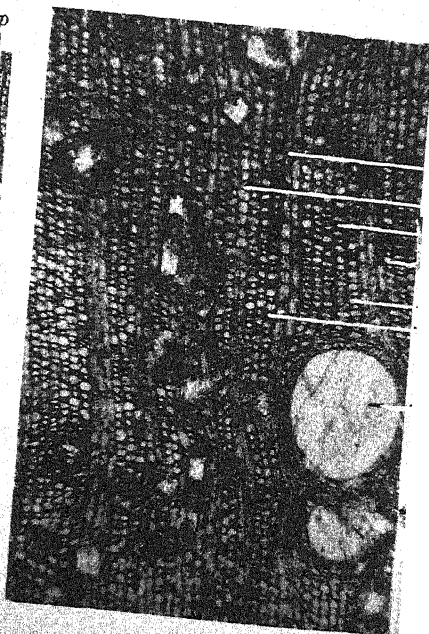
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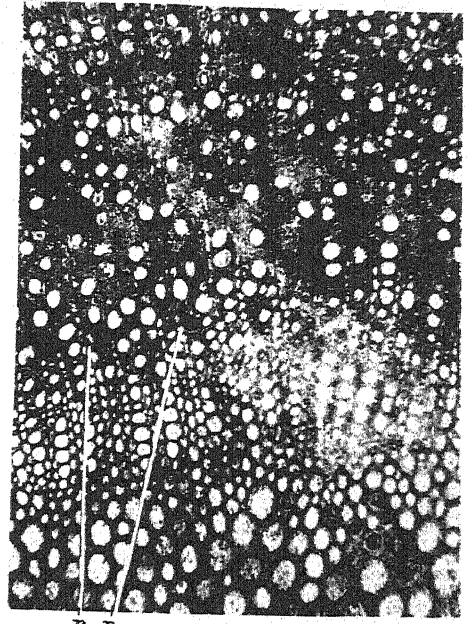
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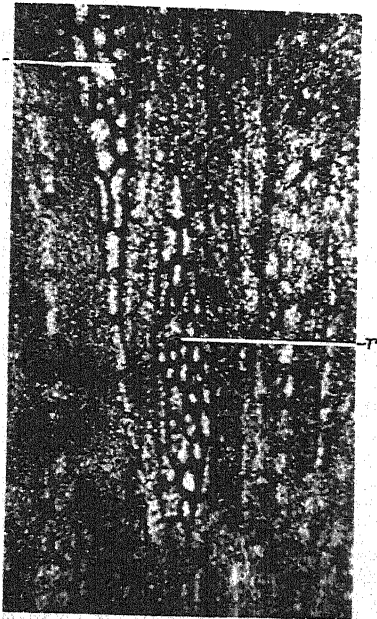
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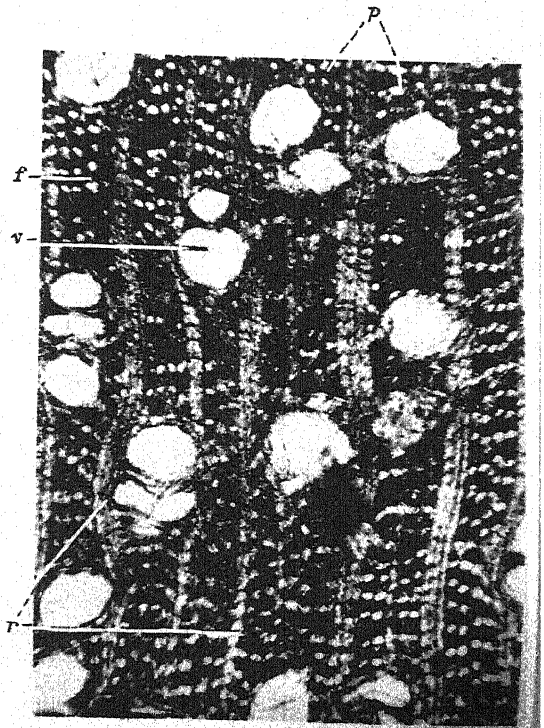
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6



7





# A Salt-marsh Form of *Fucus ceranoides*, L., from Llanbedr, Merioneth.

BY

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AND

E. H. CHATER.

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With Plate XXX and five Figures in the Text

TWO miles south of Harlech in the county of Merioneth, is the estuary of the river Artro. The curious shape of this estuary, as seen in Text-fig. 1, is due to there having been formerly an exit for the river, about a mile south of the present one. This was closed by sand dunes some years ago, and the former channel leading to this exit is now represented by the lobe of the tidal estuary to the south. At an intermediate period, both exits were open, this accounting for the name of 'Mochras Island' which is still in use for the area of cultivated land between the present exit and the site of the old one.

Salt marsh (shaded areas in Text-fig. 1) is developed near Llanbedr and Pensarn Station, and in the southern lobe near Mochras.

The distribution of the halophytic vegetation in the estuary is shown on the map (Text-fig. 2). On the Llanbedr marshes the plant communities are as follows:

(1) *Salicornia* zone.

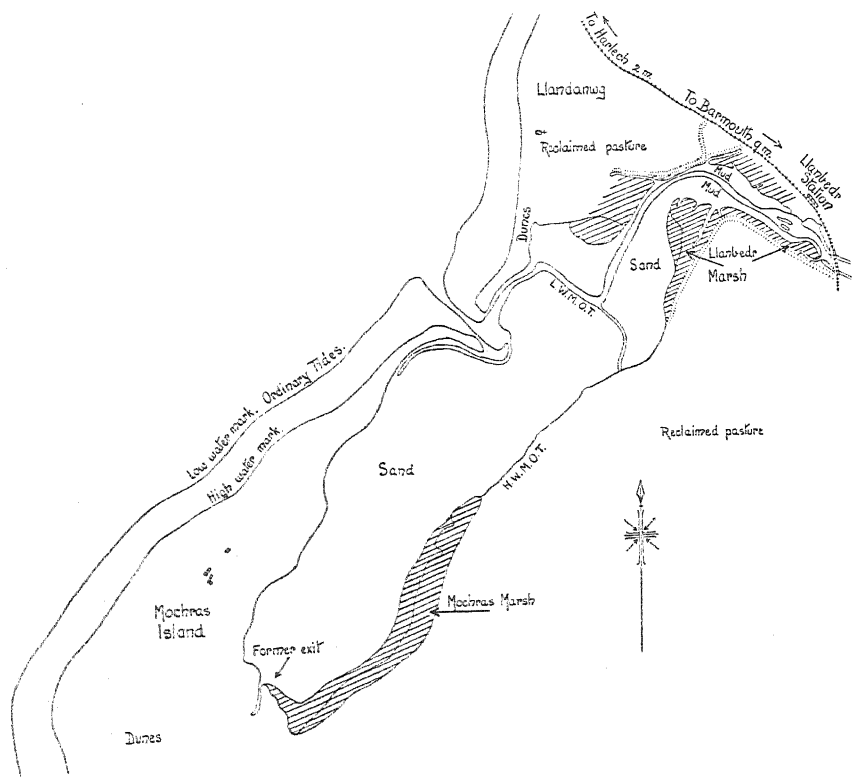
An open community of *S. herbacea*, L., present only at the west end of the Llanbedr marsh, which is rather sandy.

*Suaeda maritima*, Dum. is occasional, and a little *Glyceria maritima*, Wahl., is also present towards the upper limit of the zone, where it spreads from tufts.

(2) *Glyceria-Armeria* zone.

*Glaux maritima*, L., is frequent, and *Triglochin maritimum*, L., and *Cochlearia anglica*, L., occur as occasionals. In the higher parts *Bostrychia*

*scorpoides*, Mont., forms tufts through which the *Glyceria* grows. These tufts remain ungrazed and are conspicuous on the otherwise closely cropped sward. The upper parts of this community have an almost continuous



TEXT-FIG. 1. Outline map of the Artro estuary showing the distribution of salt marsh (shaded).

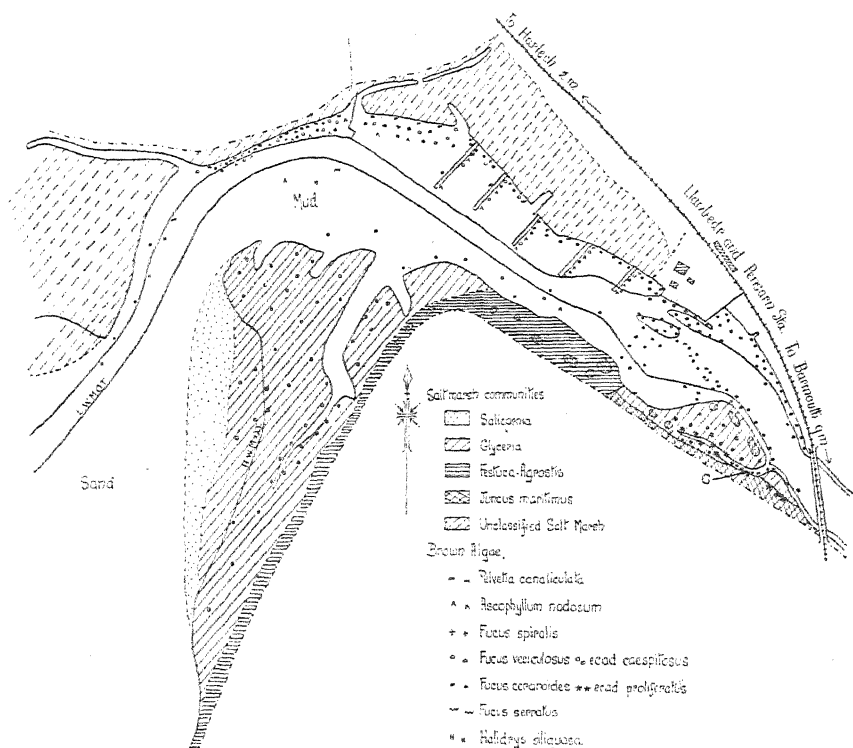
carpet of *Enteromorpha compressa*, Grev., on the surface of the substratum, while in the lower parts, *Fucus vesiculosus*, L., ecad *caespitosus*, Baker and Bohling, becomes co-dominant with *Glyceria*, forming a close sward.

In this area a number of pans are present, many of which are obviously channel pans connected by wet depressions. In these latter (Pl. XXX, Fig. 1), of the Phanerogams, *Glyceria* alone survives in any quantity. The ecad *caespitosus* of *Fucus vesiculosus* assumes a larger and more spreading habit, and gives place in the lower parts to the form of *Fucus ceranoides*, L., which will be described later on. This form also occupies the margins of the pans (Pl. XXX, Fig. 6), as well as occurring in the otherwise bare mud immediately beneath the *Glyceria* which spreads along the margins of the shallow creeks (Pl. XXX, Fig. 2).

At the east end of the marsh there occur isolated clumps of dense



*Juncus maritimus*, Lam. (Pl. XXX, Fig. 3), in the margins of which the other species of the *Glyceria-Armeria* community may be found, with the exception of the marsh form of *F. ceranoides*. *Juncus maritimus*, Lam., also



TEXT-FIG. 2. Map showing the distribution of the halophytic vegetation on the Llanbedr marsh and in the adjacent channel.

forms a continuous belt immediately above the creek, and in the wetter parts of this the marsh form of *F. ceranoides* occurs.

### (3) *Agrostis-Festuca* zone.

The main part of this zone is approximately 12-18 in. higher than the *Glyceria-Armeria* zone, and is also closely grazed. Over the greater part *Agrostis alba*, L., v. *maritima*, Meyer, is the dominant. *Festuca rubra*, L., *Glaux maritima*, L., and *Armeria maritima*, Wittd., are frequent, and *Triglochin maritimum*, L., and *Plantago coronopus*, L., are occasional. In the upper part, however, *Agrostis*, though still frequent, has ceased to be dominant, being largely replaced by *Juncus Gerardi*, Lois., and *Festuca rubra*, L., the latter being probably dominant. *Plantago Coronopus*, L., is here also abundant. *Glaux* and *Spergularia* sp. are frequent, while the

presence of *Taraxacum* and *Trifolium* spp. indicate the lower salinity due to the fresh surface water from the river at high tide.

*Plantago maritima*, L., and *Aster Tripolium*, L., appear to be restricted to the west end of the marsh, where the former is abundant in the *Agrostis-Festuca* zone, and the latter frequent in the *Glyceria-Armeria* zone. Here, also, in the lower parts, *Fucus vesiculosus* ecad *caespitosus* shows a tendency to assume a 'muscoid habit', its tufted apical proliferations being terete.

It is a different type of marsh which occurs in the extreme south corner of the estuary, and near Mochras. Being adjacent to the dunes, this area is distinctly sandy and occupied by a *Glaux-Plantago maritima* community on coarse sand on the tidal side, and a *Juncus maritimus*, Lam., zone on the more silty and somewhat waterlogged landward side. Here the *Juncus* is not dense, but is associated with a vegetation of mixed halophytes, amongst which *F. vesiculosus*, L., ecad *muscoïdes*, Baker and Bohling, forms in parts a dense undergrowth. *Pelvetia canaliculata*, Deene and Thur., ecad *muscoïdes*, Skrine, is also recorded from this area, but the marsh form of *F. ceranoides* referred to above does not occur here.

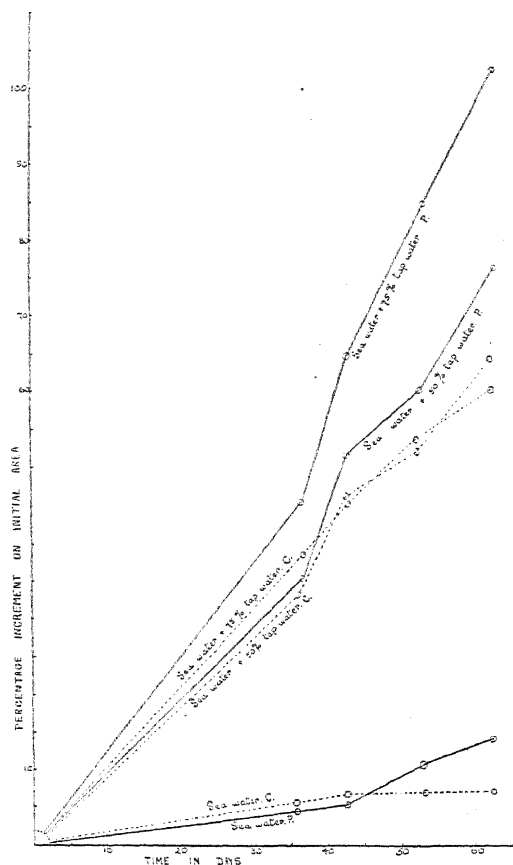
The form of *F. ceranoides* from the Llanbedr end of the estuary was formerly described as *F. vesiculosus*, L., ecad *volubilis*, Turner (4 and 7). Its appearance suggested, however, that it might be a form of *F. ceranoides*, especially in view of its papery texture and prominent midrib: sections of the thallus also showed characters of this species as will be seen later. Subsequent examinations of the area revealed a complete series of intermediate forms, occupying a shallow creek (Pl. XXX, Fig. 4, and Text-fig. 2 C), between the normal estuarine form in the river Artro and the typical marsh form at its upper limit on the marsh.

From the map (Text-fig. 2) which shows the distribution of the Fucaceae, it will be seen that the zone occupied by this marsh form is well up the estuary, where the salinity conditions will not vary much from those to which the normal form, at its zone of maximum intensity, will be subject. The restriction of the marsh form to this small area of *Glyceria* marsh, and its absence from the larger but similar *Glyceria* community further west and near the bend of the bank, is thus in accordance with the distribution of the normal form, for opposite this latter part of the marsh, *F. ceranoides* reaches its seaward limit and is very infrequent.

It has been shown (2) that the optimum conditions for the growth of *F. ceranoides* occur in places where the mean salinity is far below that of sea-water. The present form was grown in culture, and it was found that its behaviour was similar to that which might be expected of the normal *F. ceranoides*.

In an investigation into the effects of certain culture solutions upon salt-marsh forms of Fucaceae, this form and *F. vesiculosus* ecad *caespitosus* were grown in various culture solutions under similar conditions of aeration,

illumination, &c., and the growth was estimated by direct measurement of the area of the thallus by means of a planimeter used upon outlines of camera lucida drawings of a magnification of 7.7 diameters. These



TEXT-FIG. 3. Graph showing response of *F. vesiculosus* ecad *caespitosus* (C) and *F. ceranoides* ecad *proliferatus* (P) when growing in water of varying salinity.

measurements were made at intervals and expressed as per cent. increments upon the initial area.

Three of the solutions used were (1) sea-water, (2) sea-water + 50 per cent. tap-water, and (3) sea-water + 75 per cent. tap-water; and Text-fig. 3 shows graphically the different response of the two marsh forms. The curves for *F. vesiculosus* ecad *caespitosus* represent average values for the smaller normal form and the larger form characteristic of wet depressions in the marsh.

It will be seen that the difference in the rate of growth, and in the total amount of growth between the two species, increases with dilution of the sea-water, and that after sixty-three days, while *F. vesiculosus* ecad

*caespitosus* shows similar amounts of increment for sea-water + 50 per cent. tap-water, and sea-water + 75 per cent. tap-water, the increment of the marsh form of *F. ceranoides* in the more diluted solution is 25 per cent. greater than its increment in the less diluted solution, and nearly twice that shown by *caespitosus* for the same solution. Thus, a comparison between the growth-rate of plants belonging to these two ecads, grown in culture, gives a result which is in accordance with that which would be expected, were the type species themselves to be subjected to the same conditions.

*Fucus ceranoides* ecad *proliferatus*.

The occurrence of the plants has already been described. In habit they are procumbent, their branches radiating in all directions over the mud. The thallus is 5–15 cm. in length, 2–15 mm. wide, papery in texture, and the plants may easily be recognized by the number and arrangement of their proliferations (Pl. XXX, Fig. 5, and Text-fig. 4 A). As will be seen later, these proliferations arise, in the exposed portions, from hair-pits situated both on the margins and over the surface of the thallus. The normal form of regeneration for *F. ceranoides* may occur on torn or decaying portions of the thallus, especially where the latter is embedded in the mud. Receptacles occur frequently (Pl. XXX, Fig. 5 A), and at any period of the year: those observed have been exclusively female. Paraphyses project conspicuously from young conceptacles, but do not do so at a later stage of maturity. Air blisters are sometimes present, but no vesicles have been observed.

Anatomical investigation reveals the fact that the centre of the thallus, when young, contains elongated cells with brown contents such as characterize *Fucus ceranoides*. Furthermore, at a later stage, these cells break down, and the centre of the thallus then contains the brown homogeneous mass (Pl. XXX, Fig. 8) which is present in *F. ceranoides*.

As the form is distinct and its means of regeneration differs from that of the type, it is suggested that it should be called *F. ceranoides* ecad *proliferatus*, ec. nov.

*Diagnosis.*

Habit procumbent, plant spreading, 5–15 cm. in length, 2–15 mm. wide, papery in texture, thallus crinkled; hair-pits conspicuous, marginal and superficial, containing projecting tufts of hyaline hairs in the early stages, later either filled with short pigmented hairs or the bases of proliferations of the thallus. Receptacles occur, but only female conceptacles have been recorded.

Occurring on the edges of shallow muddy creeks, and in wet places amongst the lower *Glyceria* and *Juncus*. Near Llanbedr, Merioneth.

*Hair-Pit Proliferation.*

The most conspicuous feature of the plant is the production of proliferations from the hair-pits. They are produced on the wings and the margins of the thallus, and even from the sides of the midrib, in fact anywhere where hair-pits occur, except for a region of about 5 mm. in length at the growing apex. Text-fig. 4 A shows the proliferations arising from the margins of the thallus. They resemble young *Fucus* germlings when young and cylindrical, but they begin to flatten when they attain a length of 2 or 3 mm. On reaching a length of about 5 mm. the proliferations may, in their turn, give rise to daughter proliferations, so that on a large plant, several such generations may be represented. The length of time between one generation and the next is uncertain. The relationship does not appear to be an annual one, since there is no fixed period of proliferation production; they can be found in all stages of development at any season of the year. Furthermore, the distribution of the proliferations is haphazard with regard to their age and their distance from the apex of the regenerating organ.

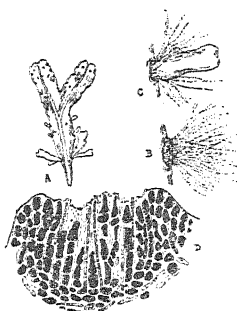
Before describing the actual hair-pit regeneration, it may be well to discuss the nature of the hair-pits themselves. Various explanations have been offered as to the initiation of a hair-pit or a conceptacle, the early stages of both being similar. Roe (5), in 1916, offered the suggestion that the hair-pit is lined by epidermal cells, the outer portions of which degenerate, while the inner portions of the same cells retain their meristematic activity, and later grow out into hairs. The hair-pit region becomes a depression by the growth of abutting tissue.

A view which has received much support from other workers is that advanced by Bower (1). The hair-pit is initiated by a single cell of the limiting layer which itself contributes nothing essential to the hair-pit, but either breaks down directly or else produces a short filament whose terminal portion degenerates, while the wall of the hair-pit is the product of the division of the cortical cell immediately below the 'initial cell' and of others adjoining it.

An explanation put forward by Simons (6) in 1906, differs in essentials from that of Bower. She maintains that the initial cell alone is responsible for the production of the hair-pit, and that the cortical cells do not contribute anything. The initial cell divides into a 'tongue cell' and a 'basal cell'. The 'tongue cell' behaves as does Bower's 'initial cell': it degenerates directly or else first divides to form a short filament. The inner product of the division of Simon's initial cell, viz. the 'basal cell', then divides and forms the entire wall of the hair-pit.

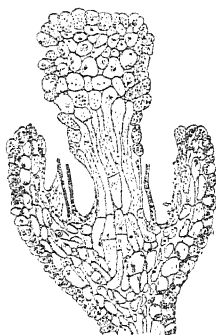
The present investigation has shown that both in *F. ceranoides* and in *F. ceranoides* *ecad. proliferatus*, there are two distinct stages in normal

hair-pit development. In the first stage, the hair-pit contains long, narrow, filamentous hairs which grow from a basal meristem; the basal cells are pigmented, but the distal portions are colourless. These arise from the floor of the hair-pit and project far beyond the aperture (Text-fig. 4 B).



TEXT-FIG. 4.

TEXT-FIG. 4. A. *F. ceranoides* ecad *proliferatus*. Portion of thallus with proliferations from marginal hair-pits. B. Hair-pit with projecting primary hairs. C. Hair-pit with young proliferation and primary hairs. D. Hair-pit with secondary hairs.



TEXT-FIG. 5.

TEXT-FIG. 5. Later stage of a proliferation from a marginal hair-pit.

With the initiation of the second stage—a further crop of ‘hairs’ arises from the floor of the hair-pit. These may be called secondary hairs (Text-fig. 4 D). They are thicker and shorter, and more densely pigmented throughout, and have a tendency to become club-shaped at their distal extremity. Except in cases of hair-pit proliferation, the secondary hairs never project beyond the surface of the hair-pit, but form a palisade-like tissue often filling the cavity of the pit, and giving an appearance not unlike the assimilating chambers of *Marchantia*. As the secondary hairs develop, the primary hairs usually die away and their remains are to be seen adhering to the apices of the new hairs. In cases where the secondary hairs are not densely packed, some primary hairs may remain amongst them. When proliferation is about to take place, the new thallus arises as a result of the congenital growth of a group of hairs situated either at the middle of the floor of the hair-pit or more laterally. All their cells retain their meristematic activity, and from this tissue the new proliferation develops. Pl. XXX, Fig. 7, shows a young proliferation consisting of a group of hairs, of which five are seen; the section is transverse through the old thallus, and longitudinal through the proliferation.

It will be seen from Text-fig. 4 C and Pl. XXX, Fig. 8, that, when a proliferation arises from a group of secondary hairs, the remainder of the pit cavity is not usually filled with these hairs, so that the long primary hairs are not all pushed off, but remain around or at the side of the young proliferation. Two, or possibly more, proliferations may arise from one pit.

At the stage seen in Pl. XXX, Fig. 7, the proliferation projects slightly

beyond the hair-pit, and its further projection is brought about by the elongation of the cells at its base. The limiting layer of the hair-pit, seen clearly in Pl. XXX, Fig. 7, has lost its identity by the time it has reached the stage shown in Text-fig. 5, so that the new thallus seems to be continuous with the inner tissues of the parent. The limiting layer of the young branch is by this time differentiated, and is still more clearly defined in subsequent stages.

While the basal cells have been elongating, the cells of the outer portion of the proliferation have been dividing in planes parallel to its long axis, so that a club-shaped structure results. This progresses still further, so that it takes on the appearance seen in Text-fig. 4 C.

So far, growth in length has been due to the activity of any or all of the cells, and not of a single apical cell. Soon, however, an apex, typical of a young *Fucus* plant, is developed, with a tuft of hairs in the apical depression. The further growth in length of the proliferation is brought about by division of the apical cell. The base increases considerably in size by cell division, forming a massive tissue embedded in the thallus,

The majority of the figures are of proliferation from marginal hair-pits, but Pl. XXX, Fig. 8, shows the origin of a branch from a hair-pit on the surface of the thallus.

In describing proliferation from the hair-pits in *Fucus vesiculosus* (a phenomenon which, as far as we are aware, has not been verified for that species), Kützing (3) describes and figures the growth of the proliferation and the subsequent obliteration of the hair-pit from which it had arisen. In *F. ceranoides* ecad *proliferatus* the cavity of the pit is not subsequently obliterated. It is persistent and recognizable as such in every case that has been examined.

#### *Morphological significance of hair-pit regeneration.*

Since the hair-pit is capable of producing a new thallus in the manner already described, the question as to its morphological nature is of interest. We have no evidence as to the relative merits of the interpretations offered by Bower (1) and Simons (6) of the appearances observed on the initiation of a hair-pit. The differences rest on the fact that Simons describes the initial cell as dividing transversely to give a basal and a tongue cell, while Bower describes the basal as being a cortical cell. According to Bower the cells lining the hair-pit are cortical in origin; according to Simons they are formed by the division of the basal cell, which is a product of a cell of the limiting layer. Bower's figures and original drawings are capable of being interpreted by Simon's explanation. The difference is that Simons has observed a division which Bower appears to have overlooked, viz. the cutting off of Bower's supposedly cortical cell from the epidermal initial cell.

Following Simon's view: a cell of the limiting layer divides into two and lies at the bottom of a depression, the wall of which has arisen from the inner product of this original cell of the limiting layer. A comparison may be made with the method of procedure on the initiation of a new apex. Here again a cell of the limiting layer, the apical cell, comes to lie at the bottom of a depression, the apical groove, the wall of which is a product of division of this cell of the limiting layer. A hair-pit, then, arises in the same way as—i.e. is morphologically equivalent to—an apex of a branch.

A further resemblance is observed, in that hairs arise from the limiting layer of the apical depression, as from the hair-pit in certain salt-marsh *Fuci*, and in the germlings of saxicolous *Fuci*.

The initial cell of a hair-pit and an apical cell, however, differ in their behaviour. An apical cell continues to cut off segments from five of its six faces, so that by its activity that region of the thallus protrudes, and a new branch is formed.

The initial cell of a hair-pit divides into two, the inner cell of which gives rise to the limiting layer of the hair-pit, and thereafter its activity ceases. Except for the outgrowth of hairs from the limiting layer, no other cell division takes place, so that that part of the thallus on which the hair-pit is situated does not form a protuberance.

The hair-pit, then, may be considered the equivalent of an apex, but does not grow out to form a branch.

We have seen, however, that in *F. ceranoides* *ecad proliferatus* the hair-pits may give rise to branches. The question then arises as to whether the branch is produced as a result of the activity of the initial cell (which we have compared with the apical cell of a shoot). Observations show that a proliferation from a hair-pit is *not* the direct product of a single cell, but is formed by the congenital growth of a group of hairs of the hair-pit. The apical cell of the proliferation, which is later differentiated, is not directly continuous with the initial cell. Of course the new apical cell, as a part of the branch which is formed from the hairs of the limiting layer of the hair-pit (itself a product of the initial cell) is thus continuous with the initial cell. But the initial cell itself does not take on the characteristics of an apical cell, and is not recognizable as such throughout the early stages of the initiation of a proliferation.

Therefore, although we can consider a hair-pit as being equivalent to an apex, we cannot say that it is a depressed apex of a potential shoot. The production of a branch by the congenital growth of a group of hairs is a unique phenomenon which, as far as we are aware, has only been described formerly by Kützing for *F. vesiculosus* in a paper which other workers have failed to confirm. It is, of course, conceivable that Kützing was actually dealing with a form of *Fucus ceranoides*.



#### SUMMARY.

A description of the plant communities of the Mochras marshes is given, and the occurrence is recorded of *Fucus ceranoides* ecad *proliferatus*, ec. nov.

The distribution of *F. ceranoides* ecad *proliferatus* is described in relation to *F. ceranoides*, L., and its growth in cultures of varying salinity is compared with that of *F. vesiculosus* ecad *caespitosus*, Baker and Bohling.

The morphology of *F. ceranoides* ecad *proliferatus* is described and a diagnosis given, together with an account of the hair-pit regeneration which characterizes the ecad.

The morphological significance of hair-pit regeneration in this form is discussed.

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#### EXPLANATION OF PLATE XXX.

Illustrating P. M. Skrine, Prof. L. Newton, and E. H. Chater's paper on A Salt-marsh form of *Fucus ceranoides*, L., from Llanbedr, Merioneth.

Fig. 1. Wet depression between two channel pans near the creek with *Glyceria* and *F. ceranoides* ecad *proliferatus*.

Fig. 2. Ecad *proliferatus* at the sides of the creek.

Fig. 3. Taken from the railway bridge looking west. The Llanbedr marsh showing patches of *Juncus maritimus*, and the creek branching off the main channel.

Fig. 4. The creek, showing, on the left, stones and mud with the intermediate form of *F. ceranoides*.

Figs. 5-8. *F. ceranoides* ecad *proliferatus*.

Fig. 5. Habit.

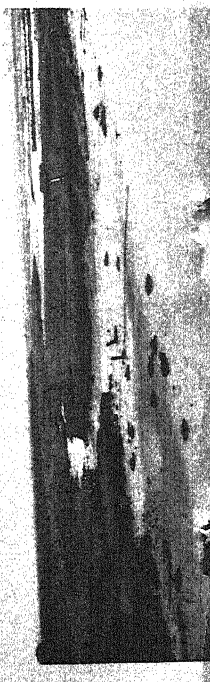
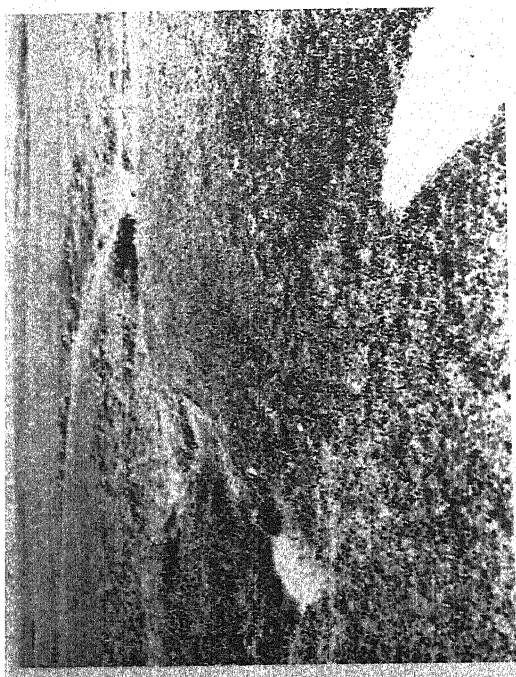
Fig. 6. Growth amongst *Glyceria*.

Fig. 7. Transverse section of thallus passing through marginal hair-pit showing an early stage in the development of a proliferation from a group of hairs.

Fig. 8. Proliferation and remains of primary hairs in a superficial hair-pit.

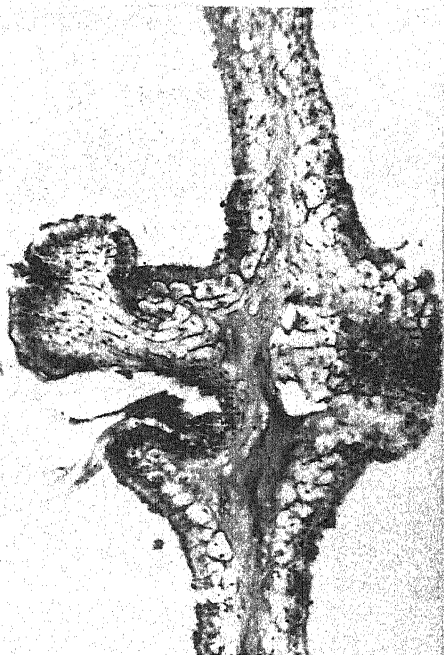


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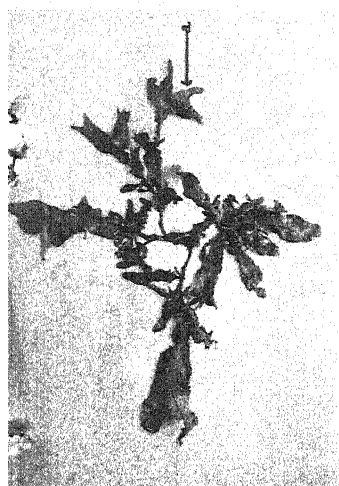




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## Studies in Growth and Differentiation.

### III. The Distribution of Calcium and Phosphate in the Tissues of '*Kleinia articulata*' and some other Plants.

BY

D. THODAY

AND

H. EVANS.

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With Plate XXXI and five Figures in the Text.

IN spite of the large amount of attention which has been devoted to succulent plants, we are far from understanding what features of their metabolism are fundamental to the succulent habit. A review of the literature by one of us (1) makes it clear that the physiological uniformity, which has often been implicitly assumed in attempts to frame causal explanations of the origin of succulence, is illusory. Even among the non-halophytes, there are exceptions to the diurnal cycle of acidity which many of them exhibit and which has come to be regarded as one of their most characteristic metabolic features.

Our study of the metabolism of *Kleinia articulata* originated from the observation that the titratable acid content of greenhouse plants was very low for a succulent and the fact that a hexose polysaccharide, inulin, was the storage carbohydrate. These features, together with the watery character of the juice, gave the impression that this succulent might differ very radically from the acid, pentosan-forming, mucilaginous type of plant usually pictured.

An account has been published (11) of an investigation into the buffer systems which operate in the extracted sap and complicate the estimation of the acid present. It was found that while malates form the chief buffer system within the range of pH normally occurring in the plant, other buffers were active above pH 6, viz. a small amount of aluminium malate, as a precipitation buffer, in extracts from young stems, and calcium phosphate in extracts from older stems.

In the extracted sap, with a pH around about 5, calcium phosphate is held in solution. During titration it is precipitated progressively after the pH

has risen above the point, about pH 6, at which the malic acid is completely neutralized to normal malate and ceases to buffer the juice. The amount of this precipitate was observed to increase very obviously with the age of the plant.

Phosphate buffering also becomes more and more marked as the stem gets older. Determinations of the titratable acid, using phenolphthalein as indicator, are too high by an amount which depends upon the amount of phosphate present. A determination of the amounts of phosphate and calcium was therefore undertaken, partly in order to obtain estimates of titratable acid corrected for phosphate buffering. (Quantitative experiments had shown that 0.01 grm. of calcium phosphate caused an increase in the titre of 0.272 c.c. N/10 baryta.)

Since, however, the pith in this stem is colourless and the assimilating tissue practically confined to the cortex, an attempt was early made to estimate separately the acid content of the cortex and of the pith. The result was to show that the outer tissues (cortex and bundle zone) are invariably more acid (as found by Richards (9) for *Opuntia*), and that in vigorous stems they show a diurnal cycle of acidity of the type normal in succulents. In the pith, on the other hand, the titratable acidity was low and the range of variation small.

It had also been observed by our colleague, Mr. N. Woodhead, that when sections were placed in alcohol a white precipitate was formed in the pith. Since calcium malate is insoluble in alcohol, the sharp localization of this precipitate raised the question of the distribution of calcium and of phosphate in the tissues. The results of a microchemical investigation were so striking that the preliminary exploration was extended to other available succulents and to some herbaceous plants.

The work to be described in this paper falls into three parts:

- (1) A quantitative macrochemical study of the calcium and phosphate content of branches and leaves of *K. articulata*.
- (2) A study, mainly microchemical, of the distribution of calcium and phosphate in the tissues of the stem.
- (3) A preliminary study of the occurrence and distribution of soluble calcium and phosphate in some other succulents and a few semi-succulents and non-succulent plants.

#### PART I. QUANTITATIVE ESTIMATIONS OF CALCIUM AND PHOSPHATE IN *KLEINIA ARTICULATA*.

METHODS. A. *Calcium* was determined by precipitation with ammonium oxalate, solution of the washed precipitate in hot dilute sulphuric acid and titration of the oxalic acid with standard potassium permanganate (1 c.c. N/10  $\text{KMnO}_4$  = 0.002g. Ca). To a measured amount of aqueous

extract was added excess of ammonium oxalate solution. After standing for some hours it was filtered through a Gooch crucible packed with purified asbestos. The calcium oxalate was washed free from ammonium oxalate, dissolved in hot dilute sulphuric acid, heated to about 60° C. and titrated. This method proved very satisfactory, duplicate analyses agreeing within 2 or 3 tenth-milligrammes of Ca in 20 c.c. of solution.

B. *Phosphate* was determined by the method of Woy, as phosphomolybdic anhydride (13). The phosphate is precipitated by means of ammonium molybdate and nitric acid as ammonium phosphomolybdate, filtered through a weighed Gooch crucible, washed and ignited until it is all converted to the bluish black anhydride. The weight of the precipitate is about twenty-four times the weight of  $P_2O_5$ . In some cases the quantity of precipitate, though just visible, was too small to weigh.

Analyses were carried out in duplicate. The duplicates agreed well within 2 to 5 per cent. according to the quantity of precipitate, the actual amounts of which varied from about 0.01 to 0.05 gm.

*Results.* Some preliminary estimations of calcium alone, made early in November, 1929, are given in Table I. Material bracketed together was taken from the same plant.

TABLE I.

*Calcium Content per 100 gm. Fresh Weight.*

*Kleinia articulata.*

	Nov. 5-7, 1929.	gram.	mg. equivalents.
1. {	i. Old leafless joints . . .	0.267	13.3
	ii. Two young joints . . .	0.099	4.9
	iii. Leaves from latter . . .	0.097	4.8
2. {	i. Very old joint . . .	0.448	22.4
	ii. Young joints . . .	0.159	8.0
3. {	i. Apical third . . .	0.136	6.8
	ii. Middle third . . .	0.151	7.5
	iii. Basal third . . .	0.182	9.1

These figures indicate that leaves and young stem are similar in having a relatively low calcium content, that the calcium increases from tip to base in a growing stem and goes on accumulating in older joints.

Subsequently determinations of calcium, phosphate and titratable acidity were made for each sample. The results are given in Table II.

These data show that *K. articulata* is very rich in soluble calcium and, in general, they tally with those in Table I.

The calcium content is least in the leaves. It increases from apex to base of a growing stem, and with increasing age of the stem. The average for young stems in Table II is 0.200 gm. per 100 gm. fresh weight, and

for old stems 0.391 grm. The oldest joint examined (two in Table I) contained 0.44 grm. per 100 grm. fresh weight.

TABLE II.

*Calcium, Phosphate, and Titratable Acid Content per 100 grm. Fresh Weight.*

<i>Kleinia articulata.</i>	Calcium.		Phosphate.		Acidity.	
	grm.	mg. equi- valents.	grm.	mg. equi- valents.	c.c. N/10 baryta	Corrected for phosphate buffering.
4. Nov. 9, 1929.						
Stem of 1928 with leafy branches of current year: 11 a.m.						
Whole plant . . . . .	0.236	11.8	0.014	0.4	12.3	11.7
5. Nov. 12, 1929.						
Three leafy shoots of current year: 9.30 a.m.						
Leaves . . . . .	0.081	4.0	0.035	1.1	19.6	18.1
Stems, upper third . . . . .	0.148	7.4	0.058	1.8	18.8	16.3
„ middle „ . . . . .	0.139	7.0	0.058	1.8	22.3	19.8
„ lower „ . . . . .	0.170	8.5	0.080	2.5	25.3	21.8
6. Nov. 14, 1929.						
Five plants with thin stems and diminutive leaves: 9.45 a.m.						
Leaves (purplish below) . . . . .	0.208	10.4	0.016	0.5	43.9	43.2
Young joints . . . . .	0.204	10.2	0.012	0.4	27.9	27.4
Older joints . . . . .	0.232	11.6	0.019	0.6	26.6	25.8
7. Nov. 15, 1929.						
Three old thick leafless joints from same pot as 6: 4 p.m. . . . .	0.405	20.2			18.8	
8. Nov. 16, 1929: 10.30 a.m.						
Leaves . . . . .	0.15	7.5	low		11.7	11.7
Joints of current year . . . . .	0.294	14.7	0.067	2.1	38.5	35.6
Old joints . . . . .	0.391	19.5	0.068	2.1	26.7	23.7
9. Nov. 22, 1929.						
Seven small young stems and four old stems from same pot: 10 a.m.						
Young stems (leaves re- moved) . . . . .	0.250	12.5	low		31.3	31.3
Old stems . . . . .	0.324	16.2	0.079	2.5	25.2	21.7
10. Nov. 23, 1929: 10 a.m.						
Two thin young pieces of stem (leaves discarded) . . . . .	0.227	11.3	low		11.3	11.3
Two old pieces . . . . .	0.322	16.1	0.0152	0.5	13.4	12.7
14. Dec. 19, 1929.						
Plants of two seasons' growth: noon.						
Stems only . . . . .	0.177	8.8	0.0614	2.0		



<i>Kleinia articulata</i> .	Calcium.		Phosphate.		Acidity.	
	gram.	mg. equi-valents.	gram.	mg. equi-valents.	c.c. N/10 baryta.	Corrected for phosphate buffering.
15. Feb. 17, 1930.						
Very young, tapering shoots of current winter's growth: 2.30 p.m.						
Leaves . . . . .	0.062	3.1	very low		2.4	2.4
Stems . . . . .	0.160	8.0	very low		2.5	2.5
16. Feb. 18, 1930.						
Shoots of 1929 summer growth still leafy and tapering: 11.30 a.m.						
Stem only . . . . .	0.260	13.0	0.0548	1.7	17.0	14.6
17. Feb. 18, 1930.						
Shoots of current winter's growth, beginning to round off: 3.30 p.m.						
	0.28	14.0	low		8.5	8.5
18. Feb. 19, 1930.						
Plants with 3 joints: 11.30 a.m.						
Joint of current winter						
Upper half . . . . .	0.106	5.3	low		12.6	12.6
Lower half . . . . .	0.157	7.8	low		14.5	14.5
Joint of 1929 summer . . . . .	0.150	7.5	0.044	1.4	18.1	16.1
Previous winter . . . . .	0.399	19.9	0.068	2.1	46.5	43.5

The phosphate content is much lower. A comparison of the columns giving milligramme equivalents shows that the bulk of the calcium in the extracted sap must be present in some other form than calcium phosphate.

The actual phosphate content is very variable. Later culture experiments indicate that it depends very much on the amount available in the soil and on other factors as well. Nevertheless certain points stand out. The phosphate content of leaves is generally very low, always less than that of the stem. In the stem the phosphate content is least at the apex and increases towards the base. It is also true on the average that old stems have more phosphate than young stems, which sometimes contained too little to estimate by the method used and with the quantity of material available; but it is not clear whether the mature stem goes on accumulating phosphate, as it does calcium, with increasing age.

On the whole, the quantitative data confirm the inferences previously drawn (11) from titration phenomena, that both calcium and phosphate accumulate in the stem. The corrections to be applied in the estimation of titratable acidity, due to the presence of phosphate in the juice, are appreciable but not very large.

	Outer tissues.	Pith.
Fairly old stem		
11.15 a.m., July 17, 1929	upper half . 11.6	6.2
	basal half . 74.0	20.5
Stem of late 1928 :		
3 p.m., Nov. 19, 1929 . . . .	23.6	9.8
Young joints :		
10 a.m., Nov. 20, 1929 . . . .	34.0	15.0
Stem of 1928 :		
10 a.m., Nov. 22, 1929 . . . .	81.0	11.8
Thin stem :		
11.20 a.m., July 31, 1929 . . . .	12.0	7.0

The following experiment (Table IV) illustrates the diurnal change of acidity in the two regions. The plants used were very healthy and vigorous, and the whole batch had been found by previous tests to be surprisingly uniform as regards their titratable acidity. Some stems were used as a whole; from others the outer tissues, including the bundle zone, were sliced from the pith.

TABLE IV.

*Titratable Acidity*

(c.c. N/10 baryta per 100 grm. fresh weight.)

Expt. 20.	June 11, 1930.	10 a.m.	10.30 a.m.	5 p.m.	5.30 p.m.
Whole stem	.	31.8		5.5	
Outer tissues	.		39.4		7.2
Pith	.		7.6		5.0
pH of juice from whole stem		4.8-5.2		5.6-5.8	

A point of immediate interest was noticed in making these titrations, namely, that in titrating extracts from the pith the end point was sharp; in other words, there was very little sign of the phosphate buffering so conspicuous in extracts of all but young shoots; whereas in the outer tissues there was extensive buffering.

The analytical data obtained by separating the tissues are given in Table V.

TABLE V.

*Calcium, Phosphate, and Titratable Acid Content of Pith and Outer Tissues*

(Per 100 grm. fresh weight.)

	Calcium.		Phosphate.		Acidity.	
	gram.	mg. equi-valents.	gram. PO <sub>4</sub>	mg. equi-valents.	c.c. N/10 baryta.	Corrected for phosphate buffering.
11. Nov. 19, 1929.						
Shoot of late 1928:						
3 p.m.						
Leaves	0.082	4.1	low		10.7	10.7
Outer tissues	0.194	9.7	0.052	1.62	23.6	21.3
Pith	0.30	15.0	practically none		9.8	9.8
12. Nov. 20, 1929.						
Stems of winter growth						
1928-9: 10 a.m.						
Outer tissues	0.139	6.95	0.084	2.62	34.0	30.3
Pith	0.274	13.7	0.035	1.10	15.0	13.5
13. Nov. 20, 1929.						
Stems of 1928 and 1929						
growth: 10 a.m.						
Outer tissues	0.071	3.55	0.001	0.03	81.0	81.0
Pith	0.151	7.55	very low		11.8	11.8

The principal result of these analyses is to show that the pith has a high calcium content and a low phosphate content relative to the tissues outside it, as well as a lower acidity. The distribution of calcium agrees satisfactorily with what would be expected from the distribution of the precipitate brought down by alcohol, assuming it to be a calcium salt.

#### *Microchemical Localisation of Calcium and Phosphate.*

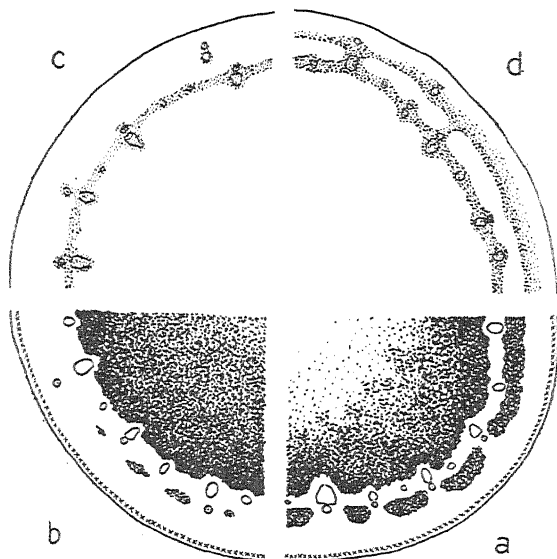
*Methods: Calcium.* In the detection of calcium the usual method has been to treat the sections with aqueous ammonium oxalate. It was found better in working with *K. articulata* to use a solution of oxalic acid in about 70 per cent. alcohol. Actually a fairly strong aqueous solution of oxalic acid was mixed with two volumes of strong alcohol. This reagent penetrates rapidly and removes the chlorophyll as well as precipitating the calcium. Sections  $\frac{1}{4}$  to  $\frac{1}{2}$  mm. thick were cut and left for a few hours in the reagent. The calcium precipitate often shows up better if the tissues are lightly stained with aniline blue (0.1 per cent. aqueous). Dark ground illumination is usually better than transmitted light.

The distribution observed was identical whether aqueous ammonium oxalate or alcoholic acid was used, and agreed exactly with the distribution of the calcium malate precipitated by alcohol alone, which was, of course, redissolved if the section were placed in water.

*Phosphate.* For the precipitation of phosphate in the cells the reagents recommended by Molisch (7) were used. The first is made by dissolving 15 grm. of pure ammonium molybdate in 100 c.c. of water by warming, and adding to this 100 c.c. of nitric acid sp. gr. 1.2 (approx. 33 per cent.). Sections, again  $\frac{1}{4}$  to  $\frac{1}{2}$  mm. thick, are cut and washed with water, and a few c.c. of the reagent, warmed to about 40° C. in a small test tube, are poured on to them in a watch-glass. When precipitation is complete they are transferred to a wash liquid containing ammonium nitrate and nitric acid. The yellow crystalline precipitate is then reduced to a bluish-black lower oxide of molybdenum by treating the section with freshly prepared 1 per cent. solution of phenyl hydrazine hydrochloride, as first advocated by Macallum (4). This technique proved very satisfactory. The sections can be cleared if necessary in alcohol, and mounted in glycerine jelly.

*Results.* The distribution of calcium was examined in a large number of plants of *Kleinia* with very uniform results (Text-fig. 1, *a*; Pl. XXXI, Fig. 1). The calcium occurs chiefly in the pith, where it is most abundant in the outer cells and decreases gradually towards the centre. It is also commonly present in well-marked groups of cells in the inner cortex. Between these two calciferous tissues is a clearly defined zone entirely devoid of calcium. We may designate it the bundle zone. It includes the

vascular bundles, interfascicular parenchyma and a few layers of perimedullary cells. The groups of calciferous cells in the inner cortex are sometimes continuous outside vascular bundles, but more often continuity is broken opposite the bundles.



TEXT-FIG. 1. *Kleinia articulata*. Diagrams of stems in transverse section showing localization of calcium and phosphate. *a*, young mature, showing distribution of soluble calcium (heavy stippling; the crosses mark the layer of calcium oxalate crystals in the collenchymatous hypodermal layer; *b*, older stem, showing similarly the increased amount of soluble calcium in the pith and the contracted patches of calciferous cells in the cortex; *c*, young mature, showing usual distribution of phosphate, in bundle zone and only around bundles in cortex; *d*, stem richer in phosphate, showing extended distribution in cortex, but leaving calciferous regions free.

The distribution changes somewhat with the ages of the stem. Very near the apex of a growing shoot the amount of calcium is relatively small, and is more or less uniformly distributed throughout the pith and more or less continuously round the inner cortex; but there is none in the bundle zone or in the outer cortex. Below the apex the outer pith cells show an increasing amount of calcium; the cortical zone becomes broken up into groups of cells which, however, also increase their calcium content. Towards the base of the shoot the calciferous cortical groups are rather smaller but more conspicuous by reason of the greater amount of calcium they contain, and a further increase in calcium is evident in the pith, extending also towards the centre. The pith, in fact, appears to be gradually filling up with calcium. Similar changes are observed if stems of different ages are compared.

In old joints the pith becomes nearly uniformly very full of calcium, even to the centre. The calciferous groups in the inner cortex on the

other hand become reduced in size (Text-fig. 1, *b*). In some old stems they consist only of three or four cells in transverse section, while in others there may be no calcium at all in the cortex.

In the leaf, calcium is less abundant and is more or less uniformly distributed, both in the colourless water tissue above and in the green assimilating tissue below.

The same material examined for *phosphate* showed a localization in the stem quite as striking as that of calcium (Text-fig. 1, *c*; Pl. XXXI, Fig. 2). Phosphate occurred practically exclusively in the bundle zone (as already defined), where calcium is absent. The phosphate precipitate is densely aggregated over the vascular bundles, but in general occurs continuously right round the stem.

In very young stems poor in phosphate the precipitate is present over the bundles and spreads for a short distance only into the adjoining interfascicular parenchyma. As the stem gets older and more phosphate is accumulated, it spreads tangentially farther from the bundles till it forms the continuous ring already described. Often some phosphate is found in close relation to leaf-trace bundles in the cortex.

This description would apply to the majority of stems examined. In some, however, which appeared to be richer in phosphate, the phosphate spread from the leaf-trace bundles tangentially in the outer cortex, forming in the extreme cases a continuous band (Text-fig. 1, *d*). The amount is never as great as in the bundle zone. Even in such stems the parts of the inner cortex where the calciferous cells occur are quite free from phosphate, and there is none in the pith.

In view of the surprising localization revealed in this microchemical examination, an attempt was made to separate the stem into three zones, cortex, bundle zone, and pith, for quantitative estimation of calcium and phosphate.

The stem selected was a very fine healthy one, 53.7 cm. long and 11 mm. in diameter. Microchemical examination of sections from apex and base showed that calcium was distributed normally and that phosphate was nearly confined to the bundle zone but occurred in association with some of the leaf-trace bundles in the outer cortex and had spread to a small extent from them to the outer cortical parenchyma on each side. The results of analysis are given in Table VI.

This experiment was successful in showing the high proportion of phosphate in the bundle zone as compared with the cortex, as well as with the pith, where, as before, it is lowest. The data agree with what the microchemical results would lead one to expect. The rather high calcium content registered for the bundle zone is due to the unavoidable inclusion with the bundle zone itself, not only of inner cortex, but some of the outermost pith cells in which calcium is most abundant.

TABLE VI.

*Calcium, Phosphate, and Titratable Acid Content of Three Zones.*

(Per 100 grm. fresh weight.)

Sept. 24, 1930: 3.30 p.m.	Calcium.		Phosphate.		Acidity.	
	grm.	mg. equi- valents.	grm.	mg. equi- valents.	c.c. N/10 baryta.	Corrected for phosphate buffering.
Cortex . . .	0.098	4.9	0.055	1.72	23.7	21.3
Bundle zone . .	0.264	13.2	0.180	5.62	35.0	27.1
Pith . . .	0.484	24.2	0.013	0.40	5.8	5.2

The additional point is of interest that the bundle zone showed the highest titratable acidity. This is in part due to the high proportion of phosphate, the correction for which brings the figure down considerably. During the titration of the extract from the bundle zone the formation of a large amount of precipitate and a very strong buffer action were observed. The end point also was much less sharply defined than in the case of pith or cortex. In the pith the end point was very well defined.

The situation revealed by these results is a very interesting one. Soluble salts of calcium and phosphate are present in some abundance; they are both accumulated as the stem increases in age, but in different tissues. The localization appears to be very sharp, the outermost of the calciferous cells of the pith, which contain the highest concentration of calcium, adjoining perimedullary cells with no calcium that can be detected by the method used, which is a very sensitive one. In the cortex there is an equally sharp contrast between calciferous and non-calciferous cells, though here we have the additional complication that, later on, cells that once contained calcium lose it.

The only other place where calcium can be detected is in the collenchymatous hypodermal layers, the cells of which, in the mature stem, contain conspicuous rhomboidal crystals of calcium oxalate.<sup>1</sup>

In view of the differences between the tissues, not only in their calcium and phosphate content, but also in titratable acidity, it is somewhat surprising to find relatively little difference in pH. Table VII gives the results of an experiment illustrating this, in which the changes in pH were followed in the pith and the outer tissues separately.

The pH determinations, except the last, were made by the rough and ready method of squeezing juice into a watch-glass and mixing with indicators. To use Gillespie's method dilution had to be resorted to in order to make a convenient volume. The results, however, suffice to show

<sup>1</sup> But see also Thoday and Woodhead (12) on the association of calcium oxalate with lignification in the woody articulation and in slender rhizomes.

that the differences between the two parts of the stem in respect of pH are small.

TABLE VII.

	Outer tissues.	Pith.
June 6, 1930: 10 a.m.: pH . . . . .	4.6-5.2	4.8-5.2
" " 4 p.m.: pH . . . . .	6.0-6.1	5.9-6.0
Darkened from 9 p.m. till June 7, 10 a.m.: pH . . . . .	4.8-5.2	4.8-5.2
Titrateable acidity, 10 a.m.: c.c. N/10 . . . . .	34.6	5.0
June 7, 10.30 a.m.: pH by Gillespie's method (2) with extracts equally diluted . . . . .	5.0	5.4

Estimation of the pH of separate tissues by several methods, including Small's range indicator method (10), gave similar results. In young stems the pH is approximately the same throughout the cross section. With increasing age, the cortex becomes slightly more acid than the pith. Gillespie's method then showed a difference up to 0.3 or 0.4 of a pH unit.

### PART III. THE DISTRIBUTION OF CALCIUM AND PHOSPHATE IN SOME OTHER PLANTS.

The unexpected and clear-cut features in the results obtained with *Kleinia articulata* led us to explore other available succulents and some semi-succulent and herbaceous plants by the same methods.

In view of the high calcium content the following succulents<sup>1</sup> were examined qualitatively for calcium and found to contain large amounts: *Kleinia neriifolia*, *Rochea versicolor*, D.C., *Crassula lactea*, *Stapelia nobilis*, *Echeveria* sp., *Crassula* spp., *Sedum* spp., *Phyllocactus* sp., *Opuntia* sp. Young Aloe leaves, though not as rich in calcium as the rest, yet contained considerable quantities. These results, together with the data provided by Hempel (3), show that a great many succulents contain a large amount of calcium in solution in their cell sap.

Further work revealed a variety of points of interest which can best be detailed for each species in turn.

#### *KLEINIA NERIIFOLIA*

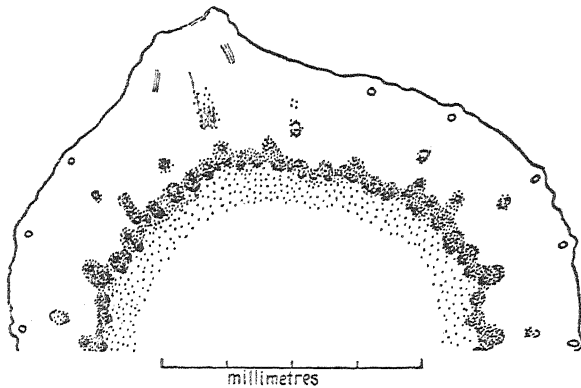
The stem of this species, which has more persistent sturdier leaves, is bigger and less juicy than that of *K. articulata* and the cells of the pith are smaller; but it is rich in both calcium and phosphate and the distribution of them is broadly similar in the two species.

In a young shoot about 1 cm. in diameter (June), the inner cortex formed a continuous calciferous zone, wider than in *K. articulata*, occupying about three-quarters of the width of the cortex, the inner margin following closely the wavy contour of the ring of bundles. The narrow bundle zone contained no calcium. The whole of the pith was calciferous,

<sup>1</sup> We are indebted to the Keeper of Botany in the British Museum (Natural History) for checking the names of some of our succulent species.



but there was a wide peripheral zone in which the cells contained only a small amount,<sup>1</sup> sharply marked off from the cells within which were very rich in calcium. In the centre the cells were again somewhat poorer in calcium.



TEXT-FIG. 2. *Kleinia neriifolia*. Young stem in transverse section showing distribution of phosphate in bundle zone, round cortical bundles and, in smaller concentration, in a peripheral zone of the pith. The distribution of calcium is complementary. In the outermost cortex neither phosphate nor calcium is found.

The phosphate (Text-fig. 2) was nearly confined, as in *K. articulata*, to the bundle zone, but a small quantity had encroached on the outer pith where the calcium content was relatively low. In the remainder of the pith there was no phosphorus; and there was none in the cortex, except close around leaf-trace bundles.

Thus in this species also the distribution of calcium and phosphate are in a high degree complementary, and we find again sharp differences between adjacent cells in respect of the composition of their sap solutes.

#### *CRASSULA LACTEA* (Soland. in) Ait.

In this plant the succulent leaves are richer in calcium than the stem and also more acid (Table VIII). Their acidity is very much higher than the stem of *K. articulata* ever shows, but their calcium content is of the same order.

Tested microchemically the stem is rather poor in calcium and there is no striking localization, most of the cells of the pith and cortex containing a small amount of it.

The leaf is much richer in calcium: all the cells contain a large amount of it. As in *K. articulata*, it is precipitated by alcohol, as well as by oxalic acid or ammonium oxalate; it is therefore present probably as calcium malate.

<sup>1</sup> In a young branch examined since in December by Miss Marian P. Roberts, B.Sc., no soluble calcium was found in this zone.

TABLE VIII.

*Calcium and Titratable Acid Content of Crassula lactea.*

(Per 100 grm. fresh weight.)

19. June 19, 1930, 9.45 a.m.		Calcium.		Acidity.
		gram.	mg. equivalents.	c.c. N/10 baryta.
Leaves	. .	0.200	10.0	90.0
Stem	. .	0.054	2.7	47.6

Abundant tannin in the stem interfered with the successful application of the microchemical test for phosphate. An extract was therefore prepared and tannin precipitated from it by means of gelatine. The resulting solution only gave a faint yellow colour with ammonium molybdate and nitric acid, and no precipitate. Therefore only a trace of phosphate was present.

The leaf, which also contains many tannin cells, gave a negative result for phosphate.

*BRYOPHYLLUM CALYCINUM.*

Tests for phosphate gave negative results in stem and leaf.

The stem was moderately rich in calcium, which was found throughout the pith and cortex, and there was no marked localization.

The leaf proved very rich in calcium, which was present in all the cells except in the vascular bundles.

*ROCHEA VERSICOLOR, D.C.*

Practically no phosphate was found by microchemical tests in either the stem or the leaf.

The stem was not very rich in calcium, which was present in small amount in most of the pith cells and also in the cortex, with no striking localization.

The leaf was very rich in calcium, which was abundant in all the cells of the mesophyll.

THE GENUS *MESEMBRYANTHEMUM.*

The species of *Mesembryanthemum* examined contain large bundles of raphides in special cells in their leaves. That these are calcium oxalate was confirmed by the usual tests. When extracts were made and tested for soluble calcium a negative result was obtained with certain species, viz. *M. inlaudens*, *M. Zeyheri*, *M. aurantiacum*. It was thought at first that little, if any, soluble calcium is present in these species. It was discovered, however, that the extracts contained a large amount of oxalic acid. The

presence of oxalic acid in such large excess would obviously be sufficient to explain the absence of calcium apart from that present as raphides. It had been noticed, however, that when sections of leaves were cut and washed in water the water became milky. This milkiess was due in part to needle crystals from disintegrated bundles of raphides, but in part to a nearly amorphous precipitate which also proved to consist of calcium oxalate. No such amorphous precipitate was found in well-washed sections, i.e. in intact cells.

In the microchemical study of this genus, therefore, the distribution of oxalic acid was studied as well as that of calcium and phosphate. The method employed was to steep well-washed sections in a hot solution of calcium nitrate.

*MESEMBRYANTHEMUM INCLAUDENS.*

Although extracts contained no calcium in solution, as already mentioned, the microchemical tests revealed certain cells very rich in soluble calcium. These were generally a little longer than the adjacent cells, though not invariably so. They were rather few in number, distributed mainly in the colourless parenchyma, though often occurring at the boundary between this and the peripheral green tissue. Oxalic acid was abundant throughout both regions; but in the water tissue there were cells, few in number, quite devoid of oxalic acid, corresponding in position to the cells containing calcium.

As the calcium in these cells was precipitated by alcohol, it is probably present as malate. The presence of malate was confirmed by qualitative tests on an extract, which indicated also that oxalic acid was present in greater amount than malic.

We may thus conclude that, in *M. inclaudent*, cells containing calcium malate occur interspersed among cells rich in oxalic acid. When extracts are made of the sap, the excess of oxalic acid precipitates the calcium from the malate.

Microchemical tests showed that this species is rich in phosphate. In many leaves, in transverse sections, phosphate was precipitated thickly over the central vascular bundle and in considerable amount through the whole of the central colourless tissue. It was present also in the green peripheral region at some points, but the amount was there much smaller. Older leaves contained more phosphate than the younger ones.

The stem, like the leaf, contained a few cells with calcium malate, interspersed among cells containing oxalic acid. The quantity of oxalic acid appeared to be much less than in the leaf. The stem, too, is rich in phosphate, which was present mainly in a central circular zone, but also in the inner cortex immediately adjacent to the central cylinder. The outer cortex contained only a minute quantity or none at all.

*M. ZEYHERI.*

Here, again, calciferous cells were found, but in rather larger numbers than in *M. inclaudens*. They were mostly distributed in the central colourless tissue, though some occurred right at the margin, partly in the green assimilating tissue. Again, malic acid was found in the extracted sap, apparently in less amount than oxalic. Oxalic acid was abundant throughout the green and the colourless parenchyma, except for certain cells, which lacked it entirely.

This species was also rich in phosphate, which occurred practically all over the leaf, even to a considerable extent in the green tissue. The precipitate was formed most thickly over the central vascular strand. The young leaves at the apex contained an appreciable amount. The stem was also rich in phosphate, which occurred in greatest abundance over the stele but also to a considerable extent in the cortex.

*M. AURANTIACUM.*

In number and distribution of calciferous cells in the leaves, this species corresponded closely with *M. Zeyheri*, as also in the occurrence of oxalic acid. The leaf was rich in phosphate, which was especially abundant towards the periphery of the colourless tissue. The green tissue contained very little phosphate. The stem was also rich in phosphate; but it was confined almost entirely to a central circular area, including the central group of vascular tissue and a narrow zone around it.

*M. VIOLACEUM.*

Quantitative analysis of extracts from the leaves of this species indicated that oxalic acid was present in smaller amount than in the previous species, and malic acid in appreciable quantity.

Microchemical examination showed that this species was richer in soluble calcium. The cells containing it were confined to the central colourless region and distributed rather irregularly. They were conspicuous by reason of their large size and the abundance of the calcium precipitate in them. Some of these cells also contained small bundles of raphides, perhaps indicating a very slow diffusion of oxalic acid or soluble oxalate into them from adjacent cells. There were, in addition, smaller cells filled with raphides. Excepting these calciferous cells, oxalic acid was generally distributed, but it appeared to be present in much smaller amount than in the species already described.

The leaf was very rich in phosphate, which appeared to be as abundant in the green tissue as in the colourless tissue within. The stem was also rich in phosphate, which was present mainly in the stele but also to a large extent in the cortex.

*M. TURBINATUM.*

In the extracted sap of this species a small amount of calcium was found, also some oxalic acid, though not a large amount, and an appreciable quantity of malic acid.

Like *M. violaceum*, this species was rich in soluble calcium and showed a similar distribution of the calciferous cells and of oxalic acid; but the latter was not as abundant. Phosphate was abundant in the central region, but practically absent in the assimilating tissue, or present only in small amount. The stem contained less phosphate than that of *M. violaceum*, but it was distributed similarly, in the central cylinder and in the adjacent cells of the inner cortex.

*M. ACINACIFORME.*

In the extracted sap of this species both calcium and malic acid were abundant, but there was no oxalic acid. Microchemical tests applied to the leaf showed that instead of the calciferous cells forming a minority, as in the previous species, only a few of the cells of the colourless tissue were devoid of soluble calcium. In accordance with this, oxalic acid was only present in a few of the colourless cells; but it was found in most of the cells of the assimilating tissue.

The leaf was very rich in phosphate, mainly located in the colourless tissue, but present also in smaller quantity in the green peripheral tissue. In the stem, which was also rich in phosphate, it was almost confined to the central cylinder, and most abundant in the pith. In the cortex it was only found in association with leaf-trace bundles.

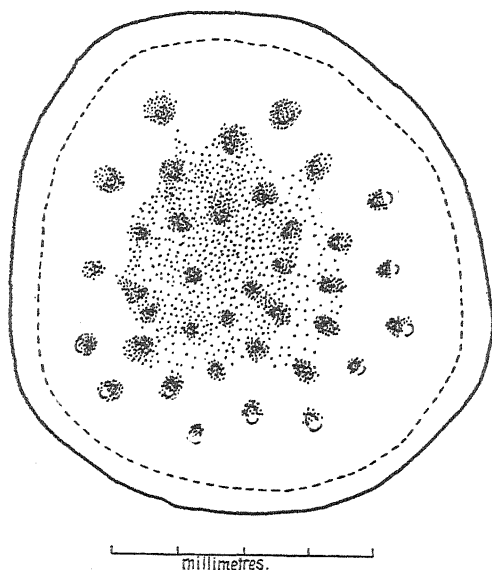
*M. EDULE.*

This species appeared to be intermediate in type between species like *M. violaceum* and *M. turbinatum*, with a minority of calciferous cells and *M. acinaciforme* in which calciferous cells are in the majority. The calciferous cells are large and conspicuous, and seem to be aggregated specially towards the three corners of the colourless central tissue. The cells in this region containing oxalic acid were smaller and formed a meshwork between the calciferous cells. Oxalic acid was found in most of the green cells. The leaf was rich in phosphate; it seemed to be present in the smaller cells containing oxalic acid, rather than in the calcium malate cells. The phosphate precipitate was specially aggregated towards the periphery of the colourless region, but was also present to a much smaller extent in the assimilating tissue.

*STAPELIA NOBILIS.*

This plant (collected by one of us at the Victoria Falls) was growing vigorously in a Wardian case in which the temperature was kept above a minimum of 16° C. The stem examined was relatively poor in calcium.

Localization was not exhibited in a marked way. The whole of the pith and bundle zone contained small amounts, it was also present to a smaller extent in the inner cortex, but none could be detected in the outer cortex.



TEXT-FIG. 3. *Peperomia tithymaloides*. Transverse section of stem showing distribution of phosphate (stippled). Soluble calcium occurs in a thicker-walled zone of tissue outside the broken line.

No oxalic acid could be demonstrated. Malate was present, but the qualitative test indicated that the quantity was small. The specimen varied from rather poor to moderately rich in phosphate, located over the vascular tissues, especially the xylem, and the interfascicular tissue. It was found in small amount at one point in the green tissue of the cortex, and also in the pith where it was closely associated with medullary phloem group.

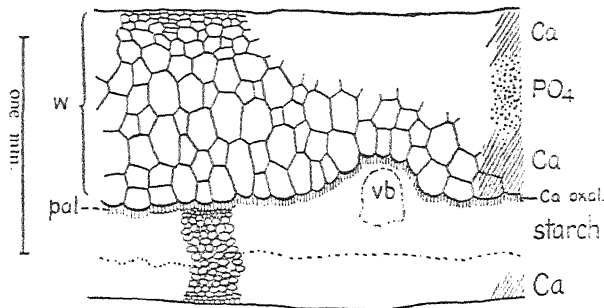
#### *PEPEROMIA TITHYMALOIDES*, A. Dietr.

This plant was selected as a semi-succulent type, with a leaf bearing a wide zone of water-tissue on its upper side.

*Stem.* The stem as a whole was not very rich in calcium, but what was present was rather strikingly distributed in a wide continuous zone of more or less collenchymatous cells in the outer cortex, where it was present in some quantity. Inside this zone a minute amount was found in some of the cortical cells, and nowhere else; but small crystals of calcium oxalate, of various forms, are present in groups in many cells of the parenchymatous ground tissue.

The stem was moderately rich in phosphate. This too, showed a definite localization (Text-fig. 3), in association with the scattered vascular

bundles. In the centre of one stem, however, it occurred also in the parenchyma between the bundles.

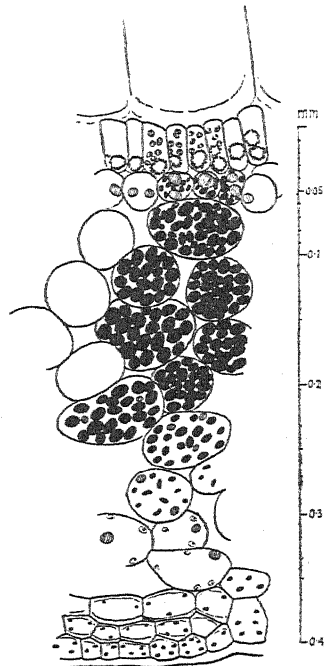


TEXT-FIG. 4. *Peperomia tithymaloides*. Diagrammatic drawing of leaf in transverse section, indicating localization of soluble calcium (Ca, shaded), phosphate ( $\text{PO}_4$ , stippled), calcium oxalate and starch. w., water-tissue; pal., palisade layer with sphaerocrystals of calcium oxalate.

**Petiole.** In the petiole the distribution of calcium was very similar to that in the stem. Also, the phosphate was closely associated with the vascular bundles, only occasionally being found over the cells immediately adjacent to bundles.

In both stem and petiole phosphate was associated more closely with the xylem than with the phloem.

**Leaf-blade.** In the blade of the leaf the distribution of both calcium and phosphate were remarkable (Text-fig. 4; Pl. XXXI, 3, 4). Calcium was present in the green mesophyll mainly in a layer of cells at the under surface, with an occasional chain of cells running across the green tissue. In the water storage tissue it was present in an upper and a lower zone separated by a middle zone free from calcium. From the calciferous zones flanges of calciferous cells projected here and there into the middle zone. Phosphate, on the other hand, was present chiefly in the water-tissue, and was there found in a middle zone of cells, with occasional irregular projections above or below. Since it is not possible to precipitate calcium and phosphate in the same section it cannot be stated with certainty that the cells of the water-tissue contain either calcium or phosphate, but never both; the observations, however, suggest this interpretation.



TEXT-FIG. 5. *Peperomia tithymaloides*. Leaf. Camera lucida drawing from the lower part of Text-figure 4, more highly magnified. Starch grains black, oil globules shaded.

Outside the water-tissue, phosphate was only found in some of the larger vascular bundles, in small amount.

Additional points of interest (Text-fig. 5) are that calcium oxalate occurs in the form of crystal clusters in the uppermost layers of the chlorenchyma (a palisade layer of relatively very small cells) and in some of the (small round) cells of the next layer; and that starch occurs as small grains in the palisade layer, as large grains abundantly in several layers below this, while in the lowest layers the amount and the size of the grains diminishes very rapidly, almost to nothing. In the lowest layers some starch may again be found.

The distribution of calcium and phosphate is at any rate largely complementary in this species, in leaves and in stems.

#### HALOPHYTIC SUCCULENTS.

##### *ASTER TRIPOLIUM.*

No phosphate could be detected either in the stem or in the leaf. Calcium was demonstrated in extracted sap by ammonium oxalate, but the amount was too small to be easily recognizable by microchemical tests.

##### *SALICORNIA.*

No phosphate could be detected. Calcium was present in specialized cells of the colourless tissue which do not differ in form from the surrounding cells. As their distribution was reminiscent of that found in *Mesembryanthemum* spp. the test for oxalic acid was applied. A minute amount of oxalic acid was present in most of the colourless cells, apart from some cells which corresponded in number and distribution with the calciferous cells.

##### *SUAEDA MARITIMA.*

No phosphate was found in the leaves of this plant. Calcium was present in small quantity in a few of the cells of the colourless water-tissue.

#### NON-SUCCULENTS.

##### *HELIANTHUS.*

*Helianthus annuus* and *H. tuberosus* were examined as representing herbaceous plants of the same family as *Kleinia*. Inorganic phosphate has already been shown by Martin (5, 6) to account satisfactorily for the buffer action of the extracted sap of the Sunflower stem in the natural range of pH.



*Helianthus tuberosus* (Autumn).

*Phosphate.* The stem of the Artichoke was very poor in phosphate compared with *K. articulata*, but the distribution had certain points of interest. The greater part of the phosphate was located in the multicellular hairs. In the upper part of the stem, where the hairs are most abundant, no phosphate was demonstrable except in the hairs. At the base of the stem, where the hairs had mostly dropped off, a few particles of precipitate occurred at intervals in the bundle zone.

*Helianthus annuus* (July).

*Phosphate.* This stem also was poor in phosphate. Near the apex it was confined to the basal cells of the hairs and, to a small extent, the cortical cells immediately below. Lower down (about the fifth internode from the base of the particular plant examined) there was a small quantity of phosphate at the centre of the pith, precipitated along the walls of the cells. At the very base, where the hairs had been shed, no phosphate could be demonstrated.

*Calcium* was found in some quantity and it was clearly localized. The rapidly growing apical part showed little or none, but lower down soluble calcium was found in increasing quantity. It first appeared in the pith distributed patchily over a central cylindrical region, in which it started to accumulate at the periphery, discontinuously at first. Proceeding downwards, the calcium increased and spread inwards and between the patches until it filled the whole cylinder. About half-way down the stem small groups of calciferous cells appeared in the cortex, and these farther down extended tangentially till they fused to form a continuous zone towards the base of the stem. Where at the base there are four main masses of xylem and the pith becomes four lobed in transverse section, the calciferous zone conformed, still leaving a rather wide perimedullary zone free from calcium.

The distribution of soluble calcium in this stem thus corresponds rather closely to its distribution in *K. articulata*. There is similar tendency for the accumulation of calcium first in the outermost cells of the calciferous tissue in the pith, followed by a gradual filling up towards the centre. The perimedullary zone without calcium is, however, much wider. The cortical calciferous zone corresponds more closely with that of *K. neriifolia*.

*PYRUS MALUS* (Young Fruit).

A young apple proved very poor in phosphate except for the ovules in which phosphate was present in some quantity. It was present to some extent in the horny wall of the loculi, but in the flesh of the fruit was only found in small amount associated with small vascular bundles and in a few

of the parenchyma cells scattered at random. Most of these cells contained some calcium, the amount of which was, however, rather small.

#### DISCUSSION.

The results described in this paper only represent the fruits of a rapid and limited preliminary exploration. They suffice, however, to show that the phenomenon of sharp localization of accumulated calcium and phosphate is not confined to *K. articulata*; and, on the other hand, that species differ in this regard.

Phosphate was found strongly localized in association with the vascular bundles in the stems of *K. articulata*, *K. neriifolia*, *Stapelia nobilis*, and *Peperomia tithymaloides*. In the leaf-blade of *Peperomia* it was confined to the middle region of the water-tissue on the upper side of the leaf.

In the species of *Mesembryanthemum* it was distributed more generally, though not uniformly, but there was no clear indication of the principles governing its distribution.

In the Crassulaceae tested and in the halophytic succulents there was practically no phosphate; in the stems of *Helianthus* very little, and that mostly in the hairs; in a young apple none in the flesh, only in the seeds and the core.

Calcium was more or less generally distributed in the parenchyma of the leaves and stems of the Crassulaceae and in the leaf of *K. articulata*. It was general though not abundant in the stem of *S. nobilis*, except that there was less of it in the inner cortex and none in the outer. Calcium was found sharply localized in *K. articulata* and *neriifolia* (stem), and in *P. tithymaloides*. In *Kleinia* and *Peperomia*, at any rate, the localization of calcium and phosphate was complementary. Perhaps the most surprising example of this complementary distribution was provided by the water-tissue of the leaf of *Peperomia*.

At this stage little more can be done than to describe the facts and point out some of the problems that they raise. Of these the most obvious is one on which considerable attention is being focused at the present time. By what mechanism can cells accumulate solutes from external fluids and pile them up in greater concentration than in the external source? In *K. articulata* the problem presents itself in the form of the accumulation of calcium malate in the cells of the pith and inner cortex, and phosphate in cells of the bundle zone. With regard to the calcium malate we have found that from excised pith, when steeped in water, exosmosis of calcium malate takes place very rapidly and the tissues become flaccid. On the other hand, exosmosis of phosphate was hardly appreciable from the outer tissues in water; and thick cross sections of young stems with little phosphate, when steeped in a 0.1 per cent. aqueous solution of  $\text{KH}_2\text{PO}_4$ , accumulated an appreciable amount of phosphate from the solution, mainly in the

bundle zone. The calcium had diffused out of the pith of similar sections almost completely after 48 hours, even in a 0.1 per cent. solution of calcium nitrate.

Clearly it is necessary to obtain further information, especially regarding the conditions which must be fulfilled for the pith to retain its calcium. We have indications that the pH of the external fluid is important. In view, however, of the mutual influence of different ions and the possible application of the principle of the Donnan equilibrium, a knowledge of the other chief ions present in the cell-sap in different tissues is also necessary before possible mechanisms can usefully be considered. This knowledge we have endeavoured to obtain, and our results in this direction will be presented in a subsequent paper. Meanwhile, there is another aspect of these results which calls for some notice. In the sharp differentiation, so well seen in *Mesembryanthemum*, between cells accumulating calcium malate and cells adjoining them without any appreciable amount of calcium and, in this genus, containing oxalic acid (or soluble oxalate), we are presented with a physiological differentiation which has often no recognizable counterpart in the morphological features of the cell. It draws attention to the fact that where differentiation of form and structure do occur, this, too, is the expression of a differentiation in metabolic processes and organization.

The physiological study of differentiation has until recently dealt with the phenomena as examples of the principle of division of labour, from the point of view of the correlation between structure and function. There has been a tendency to regard differences of chemistry as consequences of differences of function. Herein lies obviously an appreciation of one aspect of the truth.

Considered causally, however, the full assumption of a special structure and function is reached through a complicated process of development. We know very little regarding the point at which the ultimate fate of a cell is decided, the nature of the decision, or the factors which determine it.

Metabolic differentiation must be one very important aspect of the process from the earliest stage. It is, therefore, highly desirable that a systematic study of differentiation from this point of view should be undertaken. Priestley and his school have contributed data in the endeavour to read development at the apex in physico-chemical terms (8). The accumulated facts of anatomy should provide on the other hand material for an inductive approach to the principles of metabolic differentiation. Since specific differences have also their metabolic aspect it may be wise to begin with a thorough investigation of individual species rather than to attempt too soon comparative studies over a wide field.

Looking in this way at the knowledge so far gleaned concerning the stem of *K. articulata*, the facts are rather striking. In the pith are cells that

accumulate calcium malate progressively with age. In the bundle zone, besides the special elements of the vascular tissues and the resin canals, are cells that accumulate phosphate, but no calcium. The starch sheath is obviously peculiar—nowhere else in the stem is starch ordinarily found. In the inner cortex we come again upon cells storing calcium malate. These, however, unlike the pith-cells, do not continue to accumulate it. The remainder of the cortical parenchyma consists of green assimilating cells. Outside this thin-walled tissue is collenchymatous tissue, the cells of which secrete thick cellulose walls and form crystals of calcium oxalate: almost certainly this means that they themselves form oxalic acid, which is not detected in any of the other tissues. Finally comes the epidermis, which has as its peculiarity the secretion of the cuticle. Thus there is ample evidence of different types of metabolism characterized by the production of very different substances, as well as the differential accumulation of ions.

The reversibility of the accumulation of calcium in the inner cortex, however, raises the question, to what extent particular metabolic phenomena are dependent upon the particular environmental conditions of the individual cells or groups of cells, and to what extent they are expressions of deep-seated differences of constitution. It also suggests inquiry into the significance of calcium malate in the pith, and the possibility of its accumulation there also being reversible. We have evidence, too, that if phosphate is very abundant it may encroach upon the calcium areas in the cortex. The influence of variations in mineral nutrition on the accumulation of mineral ions is another obvious line of inquiry. Experimental work in these directions is in progress.

The question of the significance of calcium malate leads us back to the problem of acidity from which the present investigation started. It is evident that titratable acidity, while it may often give a reliable measure of *changes* in the amount of organic acid in the plant, is no safe guide to the total amount of malic acid present in the tissues in the form of salts. The pith accumulates malate in large amount, but its titratable acidity remains low. The old greenhouse plants which were used in our earliest experiments gave very low values of titratable acidity; but their total malate content was probably considerable, largely in the form of calcium malate. If this proves to be a carbonaceous reserve substance—and there is already some evidence that it may be—should the diurnal cycle of acidity still be regarded as of primary importance, or may it not be merely incidental? Against such a view is perhaps the greater acidity changes in vigorously active plants; but the abundance of calcium in so many succulents suggests the possibility that calcium malate is commonly used as a reserve material.

Concerning the causation of succulence, there is as yet little to be said.

The parenchymatous tissues exhibit so wide a range of metabolic types that it is difficult to conceive of a single common factor leading to general enlargement of them all. Many more data are needed, and further discussion is best postponed, at any rate till other results have been detailed in subsequent papers.

#### SUMMARY.

1. In addition to crystals of calcium oxalate in the collenchymatous hypodermal layers, calcium is abundant in the stem of *K. articulata* in solution in the cell-sap, in the pith, and in patches of the inner cortex. These calciferous regions are sharply delimited from adjoining tissues, in which no calcium can be detected by precipitation with oxalic acid.

2. Phosphate is mainly localized in the bundle zone; elsewhere it is usually limited to the close neighbourhood of leaf-trace bundles in the cortex. In stems exceptionally rich in phosphate, the phosphate extends tangentially from the cortical leaf-trace bundles, and may sometimes occur in a continuous zone, outside the calciferous inner cortex.

3. Calcium accumulates in the pith of older stems, from the periphery inwards; but the cortical patches dwindle in size and finally disappear.

4. Titratable acidity is low in the pith, highest in the bundle zone, where phosphate buffering is most marked. The sap of the pith is hardly buffered at all beyond pH 6. The diurnal cycle of acidity is very slight in the pith, but well marked in the more acid outer tissues of vigorous stems.

5. Calcium has been found in some quantity in the sap of a number of other succulents, other than halophytes.

6. Complementary localization of calcium and phosphate were conspicuously present in *K. neriifolia* (stem) and *Peperomia tithymaloides* (stem and leaf).

7. Localization of phosphate in close association with vascular bundles was observed in *K. neriifolia*, *Stapelia nobilis*, and *P. tithymaloides*.

8. Very little phosphate was found in the Crassulaceae tested, and in *Helianthus tuberosus* and *H. annuus*.

9. In *Mesembryanthemum* spp. soluble calcium and soluble oxalate are both found, in separate cells, in different proportions in different species. Mutual precipitation occurs on extraction of the sap, the composition of which indicates merely the excess of one over the other.

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## EXPLANATION OF PLATE XXXI.

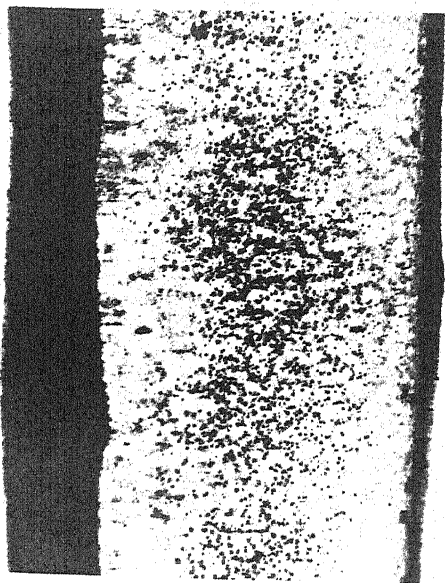
Illustrating Professor D. Thoday and Dr. H. Evans's paper On the Distribution of Calcium and Phosphate in the Tissues of *Kleinia articulata* and some other Plants.

Fig. 1. Part of a thick transverse section of a mature stem of *Kleinia articulata*, treated with an alcoholic solution of oxalic acid. The opacity in the pith and in the large patches in the inner cortex is due to calcium oxalate precipitated. In the gaps are the resin canals of principal bundles.  $\times 26.2$ .

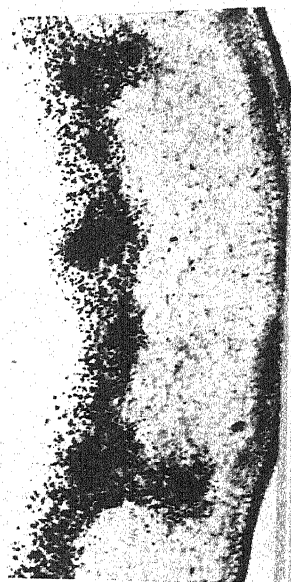
Fig. 2. Ditto, showing localization of precipitated phosphate (granules of phospho-molybdate, blackened by phenyl-hydrazine) in the bundle zone and around resin canals associated with the bundles.  $\times 26.2$ .

Fig. 3. Thick section of leaf of *Peperomia tithymaloïdes* treated with alcoholic oxalic acid, showing distribution of calcium oxalate precipitated in the water-tissue.  $\times 26.2$ .

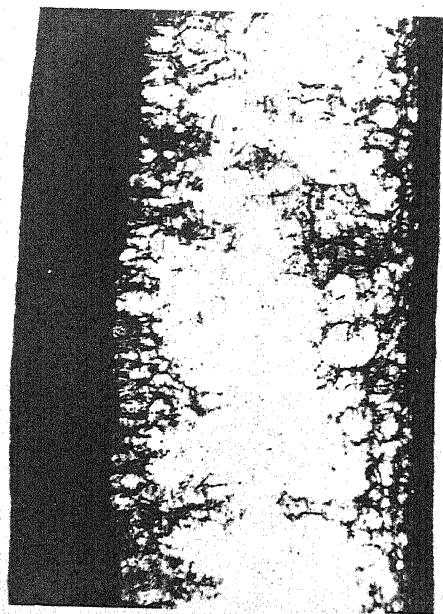
Fig. 4. Ditto, showing phosphate precipitate, more or less complementary to the calcium in distribution.  $\times 26.2$ .



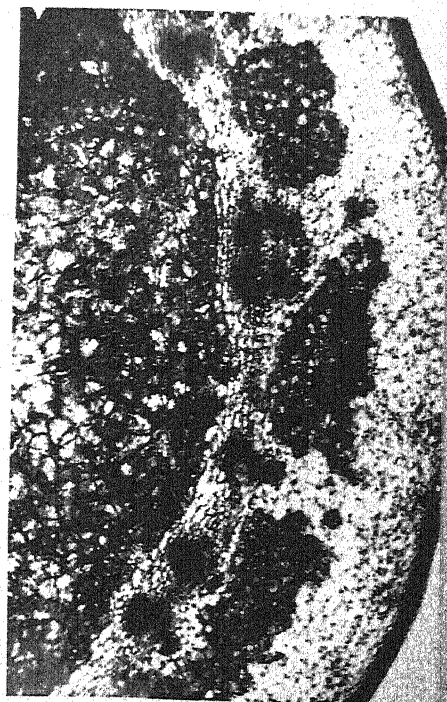
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# The Effect of Fixatives on the Prophase Stages and Heterotypic Chromosomes of *Lathyrus odoratus*.

BY

JOAN LATTER.

With Plate XXXII.

INTRODUCTORY.

IN 1926 I published an account of observations on the pollen development of *Lathyrus odoratus* (3). The figures illustrating that paper were drawn chiefly from material fixed either in Allen's modification of Bouin's fluid, or in 1 per cent. chrome-acetic solution. A few were taken from the material fixed in acetic alcohol. Examination was also made of material fixed in strong Flemming, Merkel, and Hermann's fluids, and similar phenomena were observed in all cases. Allen's Bouin gave very good general fixation and extremely clear thread stages, and from the observations a telosynaptic interpretation was placed on the mode of chromosome pairing. The attachment of the spireme to the nucleolus was also clearly observed—the point of attachment being marked by a dark-staining body lying at the periphery of the nucleolus (3, Figs. 12, 13, 14, 17, 18).

An account of meiosis in pollen mother-cells in *L. odoratus* has since been published by Maeda (5), which differs in certain respects from mine, notably in interpreting the method of chromosome pairing as parasynaptic. Also he denies that a connexion between the spireme and the nucleolus is of general occurrence and makes no mention of a deeply-staining body within the nucleolus. He observed bivalent chromosomes of many types of configuration, strikingly different from the compact rather structureless bodies obtained in my material.

The fixatives used by Maeda were Navashin's fluid and the Bonn modification of Flemming. In both cases material was first treated with Carnoy's mixture for a few minutes.

On account of these differences in the material observed by Maeda and by myself, it was thought desirable to reinvestigate the meiotic stages of *L. odoratus*, and in the summer of 1930 I obtained fresh collections of buds, using Maeda's methods of fixation, and also fixed some material again in Allen's Bouin for comparison.

## OBSERVATIONS.

The buds fixed in Allen's Bouin showed prophase stages in every way comparable with those examined five years previously. Unfortunately not many buds showed division stages, but those obtained again revealed the chromosomes as rather compact and clumped bodies with no obvious bivalent structure.

The material fixed in Navashin's fluid or the Bonn Flemming, with previous treatment in Carnoy, showed differences in the prophase stages and particularly in the structure of the metaphase and early anaphase chromosomes. In general, the Carnoy-Navashin and Carnoy-Flemming fluids do not give threads of such sharp definition as does Allen's Bouin. The threads appear more granular, and on that account are more difficult to trace with certainty. Contrary to Maeda's observations I have again found association of a loop of the spireme thread with a deeply-staining nucleolar body at the periphery of the flattened nucleolus (Fig. 1).

In the post-synaptic thread stages, when the nucleolus lies flattened against the nuclear membrane, there appears to be slight parallel approximation of some portions of the spireme. A consideration of Maeda's account of meiosis in *L. odoratus* leads one to doubt whether he has described a case of true parasynapsis. For parasynaptic pairing to take place, it is necessary that the spireme should not be a single continuous thread at the onset of meiosis. Maeda makes no definite statement as to the continuity or discontinuity of the spireme. This point is not clear from his figures, and his description of the 'segmentation of the spireme occurring step by step as it loosens from the synizetic knot' suggests that at an earlier stage he considers it non-segmented, i.e. continuous. This point is extremely difficult to determine on account of the fine granular character of the thread. In my material there are certainly no obvious free ends (Pl. XXXII, Fig. 1), but in the light of later prophase stages, which (using Carnoy-Flemming and Carnoy-Navashin fluids) agree with a parasynaptic interpretation, it is doubtful whether the continuity is real.

Somewhat later the spireme appears as seven loops of still somewhat fine granular threads (Pl. XXXII, Fig. 2). In places the threads show their dual structure, i.e. two half-chromosomes separated by the homotypic split. This stage is exactly comparable with those previously obtained with Allen's Bouin fixative (3, Figs. 20, 21).

Each loop (Pl. XXXII, Figs. 2, 2 a) represents a bivalent chromosome. A parasynaptic or telosynaptic interpretation depends on the interpretation of the association of threads at the ends of the loops. Loop 'd' is clearly formed by an end-to-end association of its members. Such a loop could have been formed by the segmentation of a continuous spireme into seven parts, and the subsequent uniting of the free ends of the members of a pair.

Loops 'e', 'f', 'g', suggest a similar method of formation, but show closer fusion of the two members of each pair at one end of the loop, while loop 'b' might be an earlier stage of such association. Loop 'c' supports a parasynaptic view, the free ends of the two arms and parallel association of the ends can be seen. Also the members of loop 'a' are parallel for a great part of their length, but one end was unfortunately obscured behind the nucleolus.

This stage of seven loops is slightly earlier than that which was previously named 'brochonema' where the seven loops radiate out in the nuclear cavity. The loops at brochonema are sharply defined in material fixed in Allen's Bouin. Maeda does not find the brochonema condition. Stages which obviously correspond to brochonema are found in my Carnoy-Flemming material, but the more coarse and granular appearance of the threads somewhat obscures the exact limits of each loop (Pl. XXXII, Fig. 3).

The apex of each loop was formerly interpreted as the point of end-to-end union of the two members of a pair. If the condition is parasynaptic, the apex of each loop is a region of parallel association of the two members.

Progressive pairing, thickening, and contraction of threads leads to the stages shown in Pl. XXXII, Figs. 4 and 5, which, provided the strands have at all stages been free, seem definite evidence for parasynaptic union. Cross-points can be seen between the two members of some bivalents, and the dual nature of the univalents may be still apparent. In many cases the free ends of the two components of the bivalents are seen. There is no typical zygotene stage.

Further condensation leads to diakinesis (Pl. XXXII, Figs. 6, 7, 8). The forms assumed by the bivalent chromosomes resemble those previously obtained by treatment with Allen's Bouin and other fixatives and show similarity to those figured by Maeda in *L. odoratus* and by Fisk in *L. tuberosus* (1) using Carnoy's and Flemming's fluids, and the aceto-carmine smear method. Connexions are sometimes present between the bivalents. If the spireme is at first a continuous structure, such connexions would be the result of incomplete segmentation of a telosynaptic spireme. This interpretation I formerly placed upon them (3, Fig. 26). If parasynapsis has occurred, these connexions are probably the result of former interlocking of pairs. There is no conclusive evidence of interlocking in diakinesis, but if this process occurs, then the fine thread stage which earlier appears to be a continuous spireme, must really consist, in part, of interlocked pairs of segments. In the case of bivalents which lie free at diakinesis, either their two members have paired without becoming entangled with other pairs, or earlier interlocked pairs have opened out and have become free of one another. In *Lathyrus* it seems that the pairing, usually at least, occurs without interlocking.

Gates and Goodwin (2) have recently shown that what was formerly regarded as a continuous looped spireme in *Oenothera* probably consists (in *Oc. purpurata* and *Oc. blandina*) of interlocked chromosome pairs in an uncondensed condition, and that in such forms there is apparently no continuous spireme at any stage. Such a condition may obtain also in *Lathyrus*. The point is very difficult to determine by direct observation, but the evidence of parasynapsis which has been found at later prophase stages indicates that probably the earlier spireme is a discontinuous structure.

The most striking differences in appearance which are obtained by the use of different fixatives are seen at metaphase and early anaphase. Buds fixed in acetic alcohol, chrome-acetic or Allen's Bouin showed metaphase and early anaphase chromosomes as compact and structureless bodies. Their bivalent nature at metaphase was often completely concealed, as also was the dual nature of the separating chromosomes at anaphase (3, Figs. 32-9). My observations of metaphase on material fixed in Carnoy-Navashin and Carnoy-Flemming fluids agree with those of Maeda (4) and (5). The chromosomes show several types of configuration and the spiral structure, especially of the rod-shaped gemini, is clearly revealed (Pl. XXXII, Figs. 9, 10, 11).

#### CONCLUSIONS.

The method of fixation has a very marked effect on the appearances observed. This is very striking at the division stages, but perhaps of more significance at prophase, where, according to the fixative used, one obtains either a completely telosynaptic or partly parasynaptic story from the preparations. It appears that some of the pairs are truly telosynaptic, formed by a portion of thread representing a pair of chromosomes end-to-end, the two arms of which have come to lie parallel, thus giving a pair of free tips at one end and a loop at the other. Other pairs appear to be truly parasynaptic, formed by the lateral approximation of two threads each representing a univalent chromosome, the resulting structure being a bivalent chromosome with free tips at both ends. It would appear that a preliminary treatment with Carnoy's fluid is the deciding factor in obtaining fixation of *L. odoratus* without complete fusion and condensation of chromatic material.

The condition in *L. odoratus* thus appears to be

(1) that the early split in the thread must be regarded as the homotypic split and not the approximation of parallel chromosomes.

(2) In the later prophase stages some pairs seemed to be formed in a telosynaptic manner, by end-to-end union of two arms of a loop, while

(3) other pairs show parasynaptic union, the parallel association first occurring at the ends of the paired strands.

I wish here to express my thanks to Professor R. R. Gates, for his kindly interest in this work.

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#### EXPLANATION OF PLATE XXXII.

Illustrating Miss Latter's paper on The Effect of Fixatives on the Prophase Stages and Heterotypic Chromosomes of *Lathyrus odoratus*.

All the figures were drawn with a camera lucida under a 2 mm. oil imm. Zeiss N.A. 1.4 with comp. oc. 15. Magnification  $\times 2,200$ .

All drawings are taken from material fixed either in Carnoy-Flemming or Carnoy-Navashin fluids.

Fig. 1. Post synzytic thread showing slight parallel approximation of strands and connexion to the nucleolar body.

Fig. 2. The spireme in the form of seven loops, some showing the homotypic split.

Fig. 2a. The seven loops of the preceding figure are drawn separately, showing the relation of each to the central nucleolus. The form of the loops is discussed in the text.

Fig. 3. This stage corresponds to that previously described as brochonema. The dual nature of some of the arms of the loops is seen. Parallel association of the thickened ends of the two arms is apparent in two of the upper loops.

Fig. 4. Parallel association of thickened strands of spireme. Cross-points occur between the members of some bivalents.

Fig. 5. Early diakinesis. The free ends of the parallel univalents can be seen, and in some cases their dual nature is recognizable.

Figs. 6, 7, 8. Diakinesis. Seven ring- or rod-shaped pairs are present in the nucleus. The bivalents are sometimes connected by slender threads. The significance of these connexions is discussed in the text.

Fig. 9. Early anaphase group of chromosomes with various types of configuration.

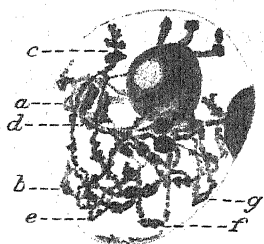
Fig. 9a. The seven chromosomes of the anaphase group drawn separately. The ring pairs separate as V's whose dual nature is seen. The rod-shaped gemini show distinct spiral structure.

Figs. 10 and 11. Bivalents of various configurations taken from three different nuclei. These are essentially in agreement with those figured by Maeda.





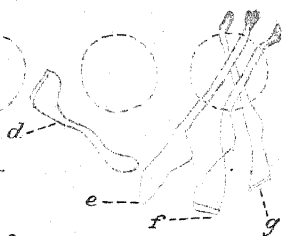
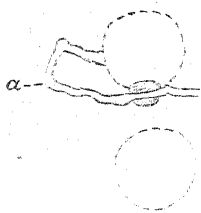
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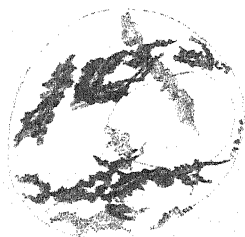
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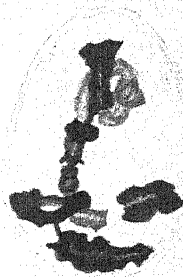
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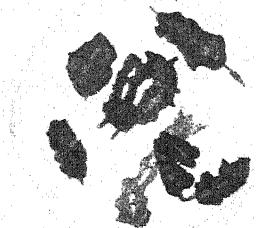
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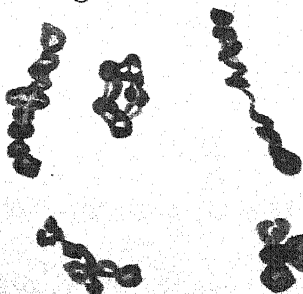
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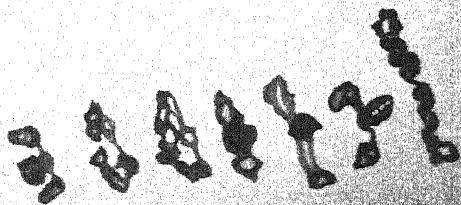
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# Studies in the Ecology of Rivers.

## II. The Microflora of Rivers with Special Reference to the Algae on the River Bed.<sup>1</sup>

BY

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(*Assistant Naturalist, Ministry of Agriculture and Fisheries, London.*)

With Plates XXXIII and XXXIV and two Figures in the Text.

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### I. GENERAL DESCRIPTION OF THE SESSILE COMMUNITY.

#### INTRODUCTION.

IN the course of investigations on the biology of the River Lark (Suffolk) undertaken for the Ministry of Agriculture and Fisheries (Butcher, Pentelow, and Woodley (11) it was found that everywhere on the bed of the stream there was a very extensive growth of algae during most part of the

<sup>1</sup> Being portion of thesis accepted for the Degree of Doctor of Philosophy in the University of London. Part I will be found in Journal of Ecology xxi, 1933.

year. As further investigations in other shallow rivers have shown these growths to be almost universal and as, further, such growths of algae have only rarely been referred to (see p. 815), it is the object of this paper first to give some account of them and, second, to examine their relation to the plankton of rivers, and to show that at least in shallow streams and rivers of the type of the Tees, Itchen, and Lark they are the most important source of river plankton.

These growths have been termed the sessile algae, and as they contribute so largely to the general biology of the stream, a method was evolved by which it was possible to estimate their nature and extent. A note of this method was first published (Butcher, Pentelow, and Woodley (9)) in 1927, and a more detailed account in 1932 (Butcher 12 a).

#### *Discussion of Method.*

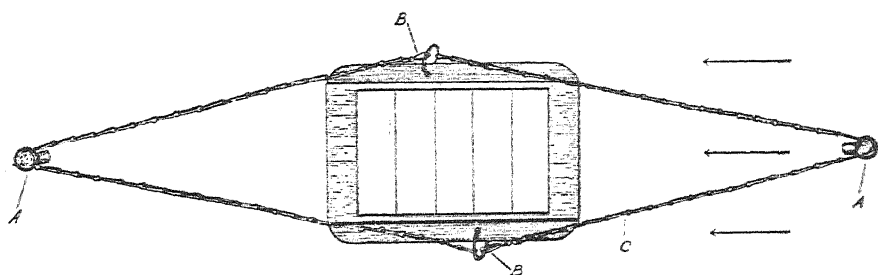
Several workers have exposed various artificial substrata for the purpose of investigating growths of microscopic organisms in water; and pieces of glass obviously suggest themselves as a convenient means of examining growths of algae, protozoa, and bacteria under the microscope without undue disturbance. Most of the other substrata used are opaque and have been abandoned in favour of glass by most of the investigators. The method of fixing the slides will, however, vary with the nature of the locality, the depth and movements of the water, and the particular aspect of the problem investigated.

Hentschel (18) appears to be the first to use this method and he employed it extensively in his investigations of micro-organisms in Hamburg harbour. Hurter (20) in his investigations on the littoral algae of Lake Lucerne employed an apparatus described by Bachmann (2). This consisted of a rectangular frame made of 'eternite' (which is a cement and asbestos boarding similar to 'uralite'), and it was anchored so that it floated near the surface; slides of glass and other material being suspended on hooks vertically from this 'buoy' to various depths in the water. This would obviously not be a suitable apparatus for use in fast-running water. Naumann (28) employed glass plates fixed in a photograph-frame for the study of iron bacteria, but gives no further details of the apparatus. Geitler (17), in his investigations on the algae of mountain streams, fixed ordinary glass micro-slides between two pieces of wood the upper of which had a heavy weight on top of it.

The apparatus used in the investigations on the Rivers Lark and Tees was evolved for use in a fast-flowing river subject to floods and containing much floating debris which would catch on to anything in the nature of a buoy or post. It was further intended for quantitative as well as qualitative investigations such as none of the above workers had attempted.

The rigid metal photographic printing frame (made by Messrs.

Houghton-Butcher, Ltd.), which was laid on the bed of the stream, held five glass slides ( $3\frac{1}{2}$  in.  $\times$  1 in.) quite firmly in any kind of current. Even in the extreme conditions of varying floods and low water found in the River



TEXT-FIG. 1. Top view of method of anchoring printing frame on bed of river. A. Iron stakes with eye. B. Spring link. C. Brass chain. Arrows indicate direction of flow of river.

Tees, such an apparatus proved successful if the frames were anchored by means of brass chain and clips to the iron stakes driven into the river-bed. The most successful type of attachment is shown in Text-fig. 1. The chains were fastened to each other by spring links. Of the five slides, four were generally used to make a general quantitative estimation by weighing the growth, while the fifth served for the identification of the species present.

As the chief aim of the Lark investigations was to discover and record the amount of seasonal variation, the frames were exposed in the river only for fourteen days so that some of the growths represented only very young stages of algae. In order to obtain the various stages in the development of the more complex types of organisms intervals of exposure varying between one and thirteen weeks are desirable.

This means of investigation is briefly referred to as the 'frame method'.

With the help of additional observations made in the River Tees it is now possible to speak more generally of the very important part played by the organisms on the river-bed. There is no doubt that extensive growths of algae as well as other organisms exist on a river-bed, and up to now these have been almost entirely neglected, although the common algae from such places have sometimes been listed (Brunnthaler (7), Limanowska (27)). The only published works dealing with these growths in detail are those of Geitler (17), who collected in a mountain stream in Austria with an apparatus referred to above, and of Fritsch (16), who collected the growths on the stones of certain streams in Devonshire during August and September. Both these workers have recorded a number of rare or new species viz :

Geitler (17).

*Hydrurus foetidus*, Kirch.

*Phaeodermatium rivulare*, Pascher.

*Chamaesiphon Polonicus* (Rost.).

*Chamaesiphon fuscus* (Rost.).  
*Chamaesiphon polymorphus*, Geitler.  
*Pleurocapsa minor* (Hansg.).  
*Homoeothrix varians*, Geitler.  
*Schizothrix semiglobosa*, Geitler.  
*Schizothrix perforans*, Geitler.  
*Phormidium autumnale* (Agh.).  
*Gongrosira* sp.  
*Pseudochantransia chalybea*, Geitler.

Fritsch (16).

*Hildenbrandtia rivularis* (Liebm.), Bréb.  
*Lithoderma fluviatile*, Aresch.  
*Chamaesiphonopsis regularis*, Fritsch.  
*Xenococcus britannica*, West and Fritsch (41).  
*Chamaesiphon pseudopolymorphus*, Fritsch.  
*Chamaesiphon ferrugineus*, Fritsch.  
*Chamaesiphon curvatus*, Nordst.  
*Pseudonocobyrsa fluminensis*, Fritsch.  
*Chroococcopsis fluminensis*, Fritsch.  
*Oncobyrsa rivularis*, Kütz.  
*Oncobyrsa cesatiana*, Rabenh.  
*Xenococcus chroococcoides*, Fritsch.  
*Gongrosira fluminensis*, Fritsch.

Similarly, I have recorded (Butcher (12)) a number of additional rare or hitherto unknown species that have been observed growing on the glass slides and have also been found among scrapings from stones or growing on *Cladophora* filaments. The list is as follows:

*Sphaerobotrys fluviatilis* sp. et gen. nov.  
*Sporotetras pyriforme* sp. et gen. nov.  
*Stigeoclonium farctum*, Berthold *rivulare*.  
*Stigeoclonium falklandicum*, Kütz. nov. var. *anglicum*.  
*Gongrosira incrustans*, Schmidle.  
*Chaetopeltis megalocystis*, Schmidle.  
*Chamaesiphon curvatus*, Nordst.  
*Chamaesiphonopsis regularis*, Fritsch.  
*Oncobyrsa cesatiana*, Rabenh.  
*Phormidium foveolarum*, Gom.  
*Heterolagynion Oedogonii*, Pascher.

That these organisms are obviously widely distributed can be seen by photographs of sample slides from the Rivers Lark (Suffolk), Itchen

(Hampshire), Tern (Shropshire), Cam (Cambridge), Skerne and Tees (Durham), all of which show similar growths (Pls. XXXIII and XXXIV).

The reason for the lack of records of these algae seems to be that the stones on the bed of a river or pond have hitherto only rarely been examined, and when any of the forms common in such places have been recorded they have been observed on such material as bits of porcelain (*Ulvella lens*, Crouan (Huber (19)) or as epiphytes on other algae, especially *Cladophora*. Washings from the stones consist chiefly of the plants most easily dislodged and often only fragments of them. Even scrapings are apt to be fragmentary and mixed with much grit. By using glass slides it has been possible to obtain many of the early stages of development of these organisms, though in all probability the mature stages of some have not yet been discovered. The growth on the slides includes a considerable number of organisms of very small dimensions many of which would be lost in the ordinary methods of shore collecting. A short account of these growths is published in the work already referred to (Butcher, Pentelow, and Woodley (11)), and as these findings have been confirmed by investigations elsewhere (chiefly on the River Tees) a more detailed account is here given.

#### DESCRIPTION OF RIVERS WHERE DATA WERE OBTAINED.

##### a. *The River Tees.*

This is one of the swiftest rivers in England and investigations have been made of the bottom-living algae by the frame method at fifteen stations on the river. Full qualitative and quantitative data have been worked out for three stations (which are called High Force, Barnard Castle, and Eryholme) and partial results of a similar kind have been obtained at a fourth station which is within tidal influence (Yarm, see Text-fig. 2).

1. *High Force* is a waterfall where the river drops 90 feet and is situated 1,000 feet above the sea-level and sixteen miles below the source of the Tees. Collections were made just above this fall where the average rate of current is high (normally 4,000–5,000 yards per hour), the water highly turbulent, and the bed composed of rocks and boulders.

2. *Barnard Castle* is fifteen miles farther downstream, and here the average rate of current is still considerable (2,500 yards per hour), the water moderately turbulent, and the bed composed of stones.

3. *Eryholme* is twenty-five miles below Barnard Castle, and at this place the river contains a considerably greater volume of water and has a slower average current (1,200 yards per hour). The water has changed completely owing to the inflow of the River Skerne which introduces hard water, heavily loaded with organic matter in the form of sewage. The river-bed is gravel and the turbulence is inconsiderable.

4. *Yarm* is ten miles below Eryholme and thirty miles from the

mouth ('South Gare'); at Yarm there is a difference of level from one to four feet between low and high tides, although the water is not saline.

Tables I, II, IX, X, and XI give the quantities of the characteristic sessile organisms.

#### b. *The River Lark.*

This river, fully described by Butcher, Pentelow, and Woodley (11), contrasts greatly with the Tees in being slow-flowing, not subject to rapid changes in water-level, and having a bed of fine gravel and mud. The frame results were obtained at Lackford where the average flow of the river was 700 yards per hour, the river-bed of gravel, and the macrophytes extremely abundant.

Yet with all these differences there is the same general character about the sessile algae as shown in Table III. This is a slight modification of the table in the paper referred to above.

#### *The Nature of the Constituent Species.*

There are three important groups of algae represented in these collections, viz. Diatomaceae, Chlorophyceae, but particularly the Chaetophorales, Myxophyceae, particularly Chamaesiphonales.

The diatoms are most abundant in the spring, but also occur spasmodically in the summer. One often very abundant and characteristic in the summer, is *Cocconis placentula*. As is to be expected, epiphytic genera are plentiful and the most characteristic representatives are *Synedra Ulna*, *Meridion circulare*, *Diatoma vulgare*, and *D. elongatum*, *Gomphonema olivaceum*, *Cymbella* spp., and *Achnanthes* spp., but *Epithemia* is very rare. Other diatoms also occur in great abundance, viz. *Amphora ovalis* f. *minutissima*, *Navicula* spp., especially *N. viridula*, *N. cryptocephala*, *N. Reinhardtii* and *N. radiosa*, *Nitzschia palea* and *N. acicularis*, *Fragilaria* spp., and *Surirella ovalis*.

Of the Chlorophyceae heavy growths of *Cladophora glomerata*, *Ulothrix zonata*, *U. subtilissima*, *Oedogonium*, and *Vaucheria* spp. are occasionally found, but the most abundant algae are those Chaetophorales previously referred to (p. 816) and which have been fully described (Butcher (12)). All of these possess the same characteristic growth, viz. a flattened disc or thallus which is firmly attached to the substratum, and sometimes from this a very poorly developed erect system arises. The most familiar examples are *Stigeoclonium* spp. but other common representatives are *Ulvella frequens* and a new genus *Sphaerobotrys*.

Of the Myxophyceae, *Phormidium foveolarum* is often abundant among the large growths, but the most characteristic are species similar to those recorded by Fritsch (l.c.) in Devon, viz. *Chamaesiphon curvatus*, *Chamaesiphonopsis regularis*, and *Oncobyrsa cesatiana*.

TABLE I (continued).

*Amount of Sessile Microflora—River Tees at Barnard Castle.*

Period of Immersion— Date to—	14 days 24. 3. 30	14 days 8. 4. 30	14 days 8. 5. 30	14 days 22. 5. 30	28 days 5. 6. 30	14 days 19. 6. 30	28 days 3. 7. 30
No./sq. cm. of sessile algae.	600	132	5,268	108,900	23,915	192,300	238,000
<i>Cyclotella Meneghiniana</i> . . . . .	—	—	—	—	—	—	—
<i>Meridion circulare</i> . . . . .	43	3	—	—	—	—	—
<i>Diatoma vulgare</i> . . . . .	210	39	214	200	30	727	—
<i>elongatum</i> . . . . .	50	7	180	—	—	—	—
<i>Synedra Ulua</i> . . . . .	5	—	90	—	—	—	2,182
<i>Acus</i> . . . . .	18	2	—	—	—	—	—
<i>tenera</i> . . . . .	130	1	232	1,300	—	—	—
<i>Ceratoneis Arcus</i> . . . . .	20	8	180	—	—	—	—
<i>Achnanthes exilis</i> . . . . .	—	2	—	—	—	—	—
<i>linearis</i> . . . . .	—	3	1,054	7,200	338	—	—
<i>microcephala</i> . . . . .	2	4	—	—	123	7,091	—
<i>lanceolata</i> . . . . .	—	—	—	127,000	—	—	—
<i>Cocconeis placentula</i> . . . . .	—	—	160	36,000	13,540	86,100	125,000
<i>Navicula viridula</i> . . . . .	—	2	393	3,700	—	—	2,542
<i>cryptocephala</i> . . . . .	—	2	—	—	—	—	—
<i>Gomphonema constrictum</i> . . . . .	—	—	—	—	—	—	—
<i>olivaceum</i> . . . . .	2	9	125	500	15	563	—
<i>geminatum</i> . . . . .	—	—	—	1,100	—	—	—
<i>Cymbella delicatula</i> . . . . .	—	2	—	—	62	—	—
<i>sinuata</i> . . . . .	76	—	—	200	—	8,181	—
<i>ventricosa</i> . . . . .	7	20	1,071	23,300	1,385	727	727
<i>Nitzschia palea</i> . . . . .	—	13	464	—	100	—	—
<i>sigmoidea</i> . . . . .	—	—	—	200	—	—	—
<i>acicularis</i> . . . . .	—	—	143	—	400	—	—
<i>dissipata</i> . . . . .	—	7	107	—	—	—	—
<i>Surirella ovata</i> . . . . .	2	1	—	—	—	—	—
<i>Scenedesmus obliquus</i> . . . . .	—	—	—	400	—	—	—
<i>Ankistrodesmus falcatus</i> . . . . .	—	—	—	—	—	—	—
<i>Ulothrix zonata</i> . . . . .	2	2	36	—	192	—	—
<i>subtilissima</i> . . . . .	5	—	54	2,700	400	—	—
<i>Cladophora glomerata</i> . . . . .	—	—	—	—	—	—	10,909
<i>Oedogonium</i> sp. . . . .	—	—	—	—	—	—	—
<i>Vaucheria</i> sp. . . . .	—	—	—	—	—	—	—
<i>Oscillatoria brevis</i> . . . . .	—	—	196	—	—	—	—
<i>Phormidium foveolarum</i> . . . . .	—	—	—	500	—	—	—
<i>Retzii</i> . . . . .	—	—	18	—	—	—	—
<i>Stigeoclonium farctum</i> v. <i>rivulare</i> . . . . .	—	—	—	—	493	—	—
<i>Uvella frequens</i> . . . . .	—	—	—	1,700	1,462	58,909	2,542
<i>Agrosira incrustans</i> . . . . .	—	—	—	—	—	—	—
<i>Aerobotrys fluviatilis</i> . . . . .	12	—	196	4,600	323	4,727	—
<i>Maesiphonopsis regularis</i> . . . . .	—	—	—	11,400	2,515	17,091	79,636
<i>Microsiphon curvatus</i> . . . . .	—	—	—	400	623	5,454	5,454
<i>Uvella cesatiana</i> . . . . .	—	—	—	600	—	545	7,254
<i>Actinotagyonion Oedogonii</i> . . . . .	—	—	—	—	—	—	—

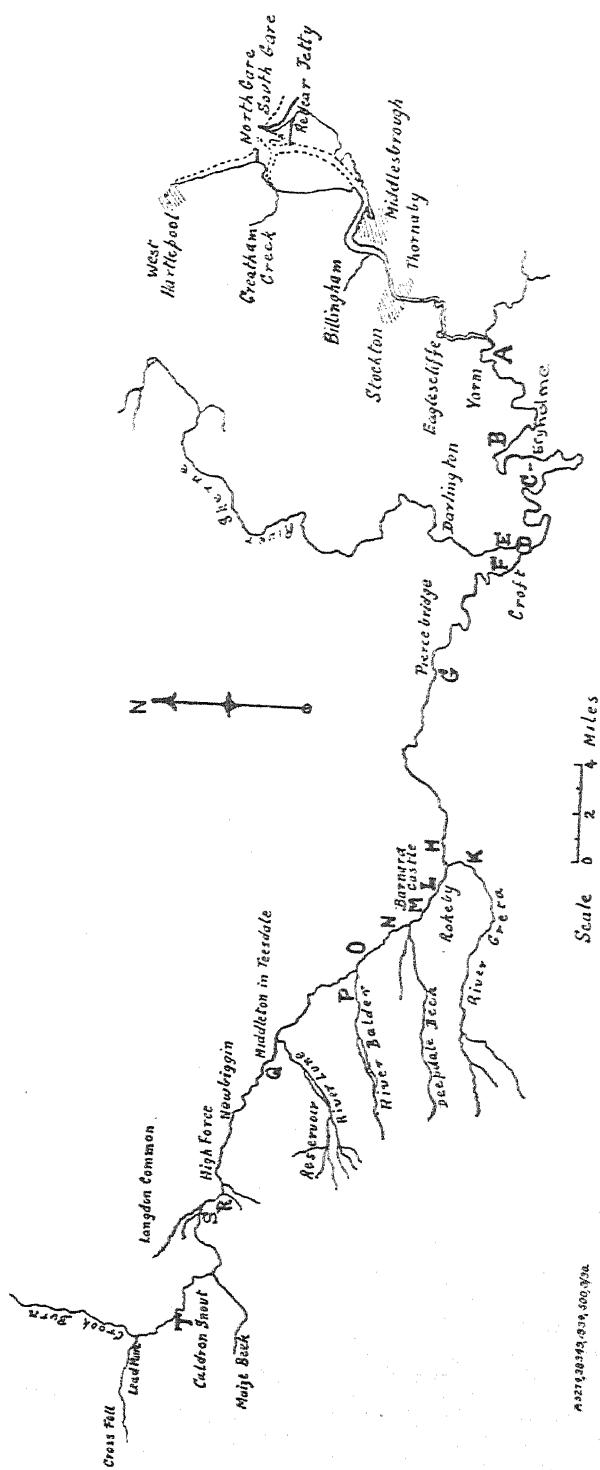
TABLE II.

*Amounts of Sessile Microflora—River Tees at Eryholme.*

Period of Immersion— Date to—	28 days 16. 7. 29	28 days 19. 8. 29	28 days 16. 9. 29	28 days 22. 10. 29	28 days 18. 11. 29	14 days 24. 2. 30	28 days 10. 3. 30	14 days 24. 3. 30
No. /sq. cm. of sessile algae.	90,900	63,100	44,300	114,600	1,300	190	17,000	125
<i>Cyclotella Meneghiniana</i> . . .	1,000	300	800	100	—	—	—	—
<i>Meridion circulare</i> . . .	—	—	—	—	—	12	250	10
<i>Diatoma vulgare</i> . . .	—	—	—	10	—	2	600	10
<i>elongatum</i> . . .	—	—	—	—	—	—	250	—
<i>Synedra Ulna</i> . . .	400	—	600	3,200	50	—	—	+
<i>Acus</i> . . .	—	—	—	—	—	—	—	—
<i>tenax</i> . . .	—	—	—	—	—	—	—	—
<i>Coscinodiscus Aratus</i> . . .	800	—	—	—	60	4	—	—
<i>Achnanthes acilis</i> . . .	—	—	—	—	—	—	—	—
<i>linearis</i> . . .	—	—	—	7,700	580	—	—	—
<i>microcephala</i> . . .	500	—	—	—	20	+	—	10
<i>Cocconeis placenticula</i> . . .	19,400	8,800	28,700	3,200	190	+	—	—
<i>Nitzschia viridula</i> . . .	—	100	300	—	160	30	5,600	50
<i>cryptocephala</i> . . .	—	—	—	—	—	6	—	—
<i>rhynchocephala</i> . . .	—	—	—	400	—	18	550	—
<i>Gomphonema constrictum</i> . . .	—	—	600	—	—	—	—	—
<i>olivaceum</i> . . .	—	—	—	—	—	24	1,400	+
<i>Cymbella delicatula</i> . . .	—	—	—	—	—	—	—	—
<i>ventricosa</i> . . .	2,100	100	300	200	40	—	500	—
<i>Nitzschia palea</i> . . .	1,300	—	400	200	130	28	1,000	10
<i>acicularis</i> . . .	—	—	200	—	+	6	500	—
<i>dissepata</i> . . .	—	—	—	—	—	—	500	—
<i>Surirella ovata</i> . . .	—	—	—	—	—	12	3,200	—
<i>Scenedesmus obliquus</i> . . .	10,700	—	—	—	—	—	—	—
<i>quadricauda</i> . . .	—	—	—	—	—	—	—	—
<i>Ankistrodesmus falcatus</i> . . .	—	—	—	—	—	—	—	—
<i>Closterium Leibleinii</i> . . .	—	—	—	—	—	—	—	—
<i>Coelastrum microporum</i> . . .	—	—	—	—	—	—	—	—
<i>Staurastrum bienianum</i> . . .	—	—	—	—	—	—	—	—
<i>Ulothrix zonata</i> . . .	—	—	—	800	—	—	100	—
<i>subtilissima</i> . . .	—	—	—	1,000	—	8	—	10
<i>Cladophora glomerata</i> . . .	100	—	300	1,000	—	—	—	—
<i>Oedogonium</i> sp. . .	—	—	—	—	—	—	—	—
<i>Vaucheria sessilis</i> . . .	—	—	—	—	—	—	—	—
<i>Oscillatoria brevis</i> . . .	—	—	—	—	—	—	—	—
<i>Phormidium foveolarum</i> . . .	—	—	+	—	—	—	—	—
<i>Sporotetrax pyriforme</i> . . .	21,900	—	1,100	—	—	—	—	—
<i>Stigeoclonium farctum</i> v. <i>reticulare</i> . . .	10,000	40,900	—	21,500	—	—	—	—
<i>fuklandicum</i> v. <i>anglicum</i> . . .	—	—	—	16,100	—	—	—	—
<i>Gomphonema incrustans</i> . . .	—	3,000	+	2,400	—	—	—	—
<i>Uvicella frequens</i> . . .	14,700	8,600	8,200	28,400	—	—	—	—
<i>Sphaerobotrys fluviatilis</i> . . .	2,700	—	+	3,800	—	—	—	—
<i>Chamaesiphonopsis regularis</i> . . .	700	—	+	2,500	—	—	—	—
<i>Chamaesiphon curvatus</i> . . .	500	—	1,100	—	—	—	—	—
<i>Oncobrya cesatiana</i> . . .	1,500	—	—	+	—	—	—	—
<i>Heterolagymon Oedogonii</i> . . .	400	700	—	—	—	—	—	—

Dominant organisms are shown in heavy figures. + = only one individual seen in count.





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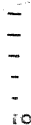


TABLE I.

*Amount of Sessile Microflora—River Tees at Barnard Castle.*

Period of Immersion— Date to—	28 days 23. 8. 29	28 days 21. 9. 29	35 days 14. 10. 29	28 days 22. 11. 29	14 days 10. 2. 30	14 days 24. 2. 30	28 days 10. 3. 30
No./sq. cm. of sessile algae.	470	203,200	137,000	13	250	341	492
<i>Ulothrix Meneghiniana</i>	—	540	—	—	—	—	—
<i>Ulothrix circularis</i>	—	—	—	—	18	32	15
<i>Ulothrix vulgaris</i>	—	360	540	2	53	112	76
<i>elongatum</i>	10	—	—	—	22	47	18
<i>Ulothrix Ulna</i>	2	3,200	—	1	—	—	2
<i>Acus</i>	7	—	—	—	9	19	6
<i>tenera</i>	—	1,400	540	—	22	54	45
<i>Ulothrix Arcus</i>	—	—	—	—	3	6	4
<i>Ulothrix exilis</i>	—	—	—	—	—	—	—
<i>linearis</i>	10	700	360	—	6	—	—
<i>microcephala</i>	7	540	84,360	—	—	12	1
<i>lanceolata</i>	—	—	—	—	—	—	—
<i>placunculata</i>	100	122,000	2,900	7	—	—	—
<i>Gomphonema viridula</i>	—	—	—	9	3	6	—
<i>cryptoccephala</i>	6	—	2,180	1	4	9	—
<i>Cymbella constrictum</i>	—	—	170	—	1	3	—
<i>olivaceum</i>	6	—	1,270	—	—	—	1
<i>Nitzschia geminatum</i>	—	—	—	—	—	—	—
<i>delicatula</i>	—	—	170	—	—	—	—
<i>sinuata</i>	—	—	—	—	—	—	—
<i>Synedra ventricosa</i>	3	—	360	—	1	2	2
<i>via palca</i>	—	—	—	1	—	—	—
<i>signoides</i>	—	—	—	—	—	—	—
<i>acicularis</i>	—	—	—	—	—	—	—
<i>dissipata</i>	—	—	—	—	2	5	—
<i>Utrixella ovata</i>	—	—	—	—	—	—	1
<i>Scenedesmus obliquus</i>	—	—	—	—	—	—	—
<i>Microcystis falcatus</i>	—	700	—	—	—	—	—
<i>Tothrix zonata</i>	—	—	—	—	—	—	1
<i>subtilissima</i>	—	—	—	—	—	—	2
<i>Radophora glomerata</i>	—	—	1,090	—	—	—	—
<i>Radophora</i> sp.	—	2,900	—	—	—	—	—
<i>Archia</i> sp.	—	—	—	—	—	—	—
<i>Scillatoria brevis</i>	—	—	—	—	—	—	—
<i>Thormidium foreolarum</i>	—	360	—	—	—	—	—
<i>Retzii</i>	—	—	—	—	—	—	—
<i>Stigeoclonium farctum</i> v. <i>riculare</i>	—	—	360	—	—	—	—
<i>Utrixella frequens</i>	—	33,450	36,100	—	+	2	—
<i>Utrixella incrustans</i>	—	—	—	—	—	—	—
<i>Phaerobotrys fluviatilis</i>	—	700	—	—	12	25	5
<i>Hammasiphonopsis regularis</i>	—	29,800	2,900	—	—	—	—
<i>Hammasiphon curvatus</i>	—	—	—	—	—	—	—
<i>Encybyra cesatiana</i>	—	—	—	—	—	—	—
<i>Laterolagynion Oedogonii</i>	330	1,720	170	—	+	2	—

NOTE.—The nomenclature in all these lists follows for the most part that of 'Die Susswasser Flora Deutschlands' (29). Dominant organisms are shown in heavy figures. + = only one individual seen & count.

TABLE II (continued).

Amounts of Sessile Microflora—River Tees at Eryholme.

Period of Immersion— Date to—	28 days 9. 4. 30	14 days 23. 4. 30	28 days 8. 5. 30	14 days 22. 5. 30	14 days 19. 6. 30	28 days 3. 7. 30	14 days 17. 7. 30
No./sq. cm. of sessile algae.	12,200	96,500	16,800,000	4,870,000	116,000	354,000	1,244
<i>Cyclotella Meneghiniana</i> . . . . .	—	—	—	7,000	3,900	16,300	30
<i>Meridion circulare</i> . . . . .	120	900	40,000	1,000	—	—	—
<i>Diatoma vulgare</i> . . . . .	120	1,450	45,000	10,000	400	+	+
„ <i>elongatum</i> . . . . .	90	3,600	160,000	16,000	—	—	—
<i>Synedra Ulna</i> . . . . .	25	550	56,000	130,000	—	—	—
„ <i>Acus</i> . . . . .	10	—	—	—	—	—	—
„ <i>tenera</i> . . . . .	140	550	+	1,000	—	—	—
<i>Ceratoneis Arcus</i> . . . . .	40	1,200	—	1,000	—	—	—
<i>Achnanthes exilis</i> . . . . .	40	—	—	2,000	—	—	—
„ <i>linearis</i> . . . . .	40	3,300	50,000	60,000	3,900	1,100	12
„ <i>microcephala</i> . . . . .	40	—	—	1,000	—	1,600	+
<i>Cocconeis placentula</i> . . . . .	80	+	86,000	90,000	7,500	75,000	370
<i>Navicula viridula</i> . . . . .	480	44,000	2,100,000	240,000	600	1,600	—
„ <i>cryptcephala</i> . . . . .	10	1,450	205,000	200	—	—	—
„ <i>rhyncocephala</i> . . . . .	—	1,450	—	—	—	—	—
<i>Gomphonema constrictum</i> . . . . .	25	—	14,000	14,000	—	—	—
„ <i>olivaceum</i> . . . . .	240	6,000	240,000	42,000	+	1,100	—
<i>Cymbella delicatula</i> . . . . .	+	—	10,000	100	200	—	—
„ <i>ventricosa</i> . . . . .	470	8,900	1,200,000	200,000	15,700	23,500	10
<i>Nitzschia palea</i> . . . . .	30	4,000	145,000	8,000	500	1,600	—
„ <i>acicularis</i> . . . . .	+	+	186,000	130,000	500	800	—
„ <i>dissipata</i> . . . . .	25	+	72,000	100	+	400	—
<i>Surirella ovata</i> . . . . .	20	6,900	4,000,000	640,000	+	+	—
<i>Scenedesmus obliquus</i> . . . . .	—	—	10,000	—	19,400	50,000	18
„ <i>quadricauda</i> . . . . .	—	—	—	—	700	2,200	—
<i>Ankistrodesmus falcatus</i> . . . . .	—	—	—	—	2,000	700	—
<i>Closterium Leibelinii</i> . . . . .	—	—	—	10,000	+	—	—
<i>Coelastrum microporum</i> . . . . .	—	—	—	—	+	300	—
<i>Staurastrum bienianum</i> . . . . .	—	—	—	—	400	+	—
<i>Ulothrix zonata</i> . . . . .	—	—	27,000	2,260,000	11,400	800	—
„ <i>subtilissima</i> . . . . .	—	9,600	—	487,000	700	—	—
<i>Cladophora glomera</i> . . . . .	120	—	—	—	700	6,500	153
<i>Oedogonium</i> sp. . . . .	—	—	—	—	4,400	15,000	44
<i>Vaucheria sessilis</i> . . . . .	—	—	—	—	+	600	—
<i>Oscillatoria brevis</i> . . . . .	—	+	—	—	—	—	—
<i>Phormidium foveolarum</i> . . . . .	—	—	—	—	1,200	7,000	14
<i>Sporotetras pyriforme</i> . . . . .	—	—	—	+	6,200	2,500	196
<i>Stigeoclonium farctum</i> v. <i>reticulare</i> . . . . .	—	—	—	14,000	12,000	70,000	17
„ <i>falklandicum</i> v. <i>anglicum</i> . . . . .	—	—	—	—	—	—	—
<i>Gomrosira incrustans</i> . . . . .	—	—	—	—	7,000	14,600	260
<i>Ulvella frequens</i> . . . . .	—	—	—	130,000	5,000	35,600	—
<i>Sphacelotrys fluviatilis</i> . . . . .	—	+	—	100,000	3,500	3,600	13
<i>Chamaesiphonopsis regularis</i> . . . . .	—	—	—	—	+	10,000	—
<i>Chamaesiphon curvatus</i> . . . . .	—	—	—	—	400	600	6
<i>Oncobyrsa cesatiana</i> . . . . .	—	—	—	—	—	—	—
<i>Heterolagynion Oedogonii</i> . . . . .	—	—	—	+	700	—	—

<sup>1</sup> This frame was turned over. Dominant organisms are shown in heavy figures. + = only one individual seen in count.

*Seasonal Variation of the Commonest Sessile Organisms—Lackford, on River Lark.*

[illegible]

Figures without brackets 1927-8. Figures as per cent. of total organisms, + = present but less than 1 per cent. Figures with brackets 1928-9. Dominant forms are shown in heavy figures.

TABLE III (continued).  
Seasonal Variation of the Commonest Sessile Organisms—Lackford, on River Lark.

Species.	July.			August.			September.			October.		
	1	(4)	(11)	18	(18)	(26)	3	(4)	(11)	18	(18)	(26)
<i>Melastira varians</i> . . . . .												
<i>Meristina circulare</i> . . . . .												
<i>Diatoma vulgare</i> . . . . .												
" <i>elongatum</i> . . . . .												
<i>Synedra Ulna</i> and vars. . . . .												
" <i>Alata</i> . . . . .												
<i>Achnanthes exilis</i> . . . . .												
<i>Cocconeis placatula</i> . . . . .												
<i>Navicula viridula</i> . . . . .												
" <i>oblonga</i> . . . . .												
" <i>cryptoccephala</i> . . . . .												
" <i>Reinhardtii</i> . . . . .												
" <i>lanceolata</i> . . . . .												
<i>Gomphonema constrictum</i> . . . . .												
" <i>parvulum</i> . . . . .												
" <i>olivaceum</i> . . . . .												
<i>Cymbella lanceolata</i> . . . . .												
<i>Anphora ovalis</i> and vars. . . . .												
" <i>Nitzschia palea</i> . . . . .												
" <i>subtilis</i> . . . . .												
" <i>acicularis</i> . . . . .												
<i>Ulothrix zonata</i> . . . . .												
<i>Cladophora glomerata</i> . . . . .												
<i>Oedogonium</i> sp. . . . .												
<i>Vaucheria</i> sp. . . . .												
<i>Stigeoclonium farctum</i> . . . . .												
<i>Gongosira</i> sp. . . . .												
<i>Ulothrix Fregens</i> . . . . .												
<i>Sphaerodolrys fluvialis</i> . . . . .												
<i>Chlorophyceae</i> indet. . . . .												
<i>Chamaesiphon curvatus</i> . . . . .												
<i>Chamaesiphonopsis regularis</i> . . . . .												
<i>Oncolysis cesatii</i> . . . . .												
<i>Heterolagynion Oedogonii</i> . . . . .												
<i>Sphaerotilins natans</i> . . . . .												

Figures without brackets 1927-8. Figures with brackets 1928-9. Figures as per cent. of total organisms. + = present but less than 1 per cent. Dominant forms are shown in heavy figures.

## SEASONAL VARIATION.

The first most obvious fact which can be deduced from these results is that there is a marked seasonal variation of the organisms concerned. There is a little difference in species between the lists from the Lark and the Tees, but the similarities are unmistakable.

This variation may be summarized as follows:

(a) *End of February to April.* The growths at this time of the year consist almost entirely of diatoms. The characteristic organisms in the Lark are *S. Ulna*, *M. circulare*, *G. olivaceum*, and *N. viridula*. In the Tees the first three are present, but the commonest organisms are *D. vulgare* and *Achnanthes* spp. in the upper river and these and *N. viridula* in the lower river.

(b) *From May to August* diatoms are rare except for *Cocconeis* which, though almost unrepresented in the *February to April* growths, is present in quantity during this period not only in the Tees and Lark but also in the Cam, Itchen, Tern, and Skerne. In addition to this diatom there is a large variety of densely aggregated green and blue-green algae, of which the commonest are *U. frequens*, *Stigoclonium* spp., *Gongrosira* spp., and *Ch. regularis*.

(c) *From the end of April to June*, in addition to the above, there appears for a short time from April to June a number of filamentous algae such as *Cl. glomerata*, *Ulothrix* spp., and *Oedogonium* spp.

(d) In addition to the above there are also to be found on the slides during the same period unattached Chlorophyceae, of which the most frequent are *Scenedesmus obliquus* and *Closterium Ehrenbergii*. These are more marked in the Tees, than in the Lark, and sometimes appear in large numbers.

(e) *From September to November* a certain number of the diatoms recorded for the spring again appear but do not displace the May to August growths of other algae.

(f) *From November to February* there is very little indeed, and the growth usually consists of occasional individuals of the commonest diatoms.

This seasonal variation of the sessile growths seems to have been first recorded in a general way by Limanowska (27), who describes similar variations in the algal flora of the Limmat, except that forms like *Ulvella* and *Chamaesiphon* are unrecorded. The resemblances to the variations of the plankton of rivers recorded by Kofoid (21), Brunnthaler (7), Schröder (37), Fritsch (14), Lemmermann (26), and many others are so marked as to leave no doubt that a true idea of seasonal variation in a river can only be obtained if the sessile organisms are studied at the same time. There are two 'major' phases of this annual cycle, viz. the diatom phase in spring

and the *Cocconeis*, *Ulvella*, *Chamaesiphon* phase in the summer. In the paper on the Lark (l.c.) the latter is referred to as the *Cocconeis-Protoderma* phase, since the thalloid alga *Ulvella* was erroneously interpreted as a *Protoderma*. The name should therefore be altered to the *Cocconeis-Ulvella* community, or perhaps it would be better to adopt Fritsch's term and speak of it as the summer-encrusting community, since many of the constituent species form films or crusts on the stones or other part of the river-bed.

### THE COMMUNITIES OF SESSILE ALGAE.

Though the study of these sessile algae has not yet proceeded very far, it is possible to draw up a number of communities on the basis of the results obtained.

#### 1. *The Ulvella-Cocconeis community.*

This community, in which *U. frequens* and *Cocconeis* spp. are usually dominant, occurs throughout the summer in all rivers so far examined by me, viz. the Lark, Tees, Tern, Itchen, Cam, and Skerne, and seems to be more or less constant in rivers with very different physiological features. This constancy applies chiefly to the two dominant plants mentioned above, as there are other species locally sub-dominant. Of these there is *St. farctum* and *Scenedesmus* in the lower Tees (see Table II), *Ch. regularis* in the Lark, and, to a lesser extent, in the Tees and Skerne.

Fritsch (16) in the Devon rivers describes several encrusting communities dominated by red and blue-green algae which he calls the *Hildebrandtia-Lithoderma* community, the *Chamaesiphon* community, and the *Phormidium* community. Geitler (17) also describes similar communities in Austria.

It seems possible that this *Chamaesiphon* community is a special development of the *Ulvella-Cocconeis* community, as the latter is apparently represented in Fritsch's collections. *Ch. regularis* and *O. cesatiana*, two algae characteristic of this community in the Lark, are also recorded as abundant in Devon.

Whether these communities in the small, swift Devon rivers can best be considered as special developments of the *Ulvella-Cocconeis* community on the whole, or whether there are special features that warrant their being considered separately, further investigations will show.

#### 2. *The Ulothrix community.*

Certain filamentous algae are abundant in the spring and early summer, and the commonest of these seems to be *Ulothrix zonata*. Other algae accompanying it are *U. subtilissima*, *Cl. glomerata*, *Vaucheria*, and

*Oedogonium* species. In some cases *Cl. glomerata* is dominant, e.g. the lower Tees.

Further work is required before the conditions under which the various species occur can be stated, but *U. zonata* seems to be the most widely distributed of all these algae, and a robust form of *Cl. glomerata* flourishes in rivers known to be polluted with organic matter. The other point to note about this community is the short season of its appearance. In England it seems to occur as a dominant only in the months of May and June, though it has been seen in September.

### 3. *The Diatom communities.*

Diatoms comprise undoubtedly the largest and most prevalent single group of algae in a river. The *Ulvella-Cocconeis* community seems to be present whatever the river, but the diatom communities vary to a great extent. Based on the Lark and Tees collections a certain number of well-marked communities may be distinguished, though when more data are acquired they may have to be modified.

(a) The *Diatoma-Gomphonema olivaceum* community is best developed at High Force on the River Tees (see Table IX), but is also obvious at Piercebridge, Barnard Castle, and Croft above the junction of the River Skerne (see Table IV). All the dominant diatoms are attached in some way. A sub-community of this is often seen in the upper river in which *Achnanthes* spp. and *U. subtilissima* are abundant.

(b) The *Navicula viridula-Cymbella ventricosa-Surirella ovata* community reaches its maximum development in April and May in the lower Tees (i.e. Eryholme, see Table II and X), but is also spasmodically represented at Barnard Castle (Table I) and Cotherstone (Table IV). *N. palea* is also a frequent member of this community, particularly in the upper river. In contrast to the *Diatoma* community above, it may be noted that the dominant diatoms possess no special means of attachment.

(c) The *Synedra Ulna* community reaches its best development in the Lark and the Cam. Two other diatoms are among the sub-dominants here, namely, *N. palea* and *G. olivaceum*, and one could sub-divide the community into two groups with these two algae respectively sub-dominant.

This only gives a preliminary outline of the communities so far brought out by these studies. There are certainly others, for instance, *Rhoicosphenia curvata* seems to be a dominant in the River Skerne, but further investigations must be made before the classification can be more definite.

### FACTORS DETERMINING THE COMMUNITIES.

It has been pointed out by Pearsall (33) that, in the Wharfe, phosphate, silicate, and probably nitrate are not subject to such extreme exhaustion as



they are in lakes (Pearsall (34)) and in the sea (Atkins (1)), and presumably are not of great importance in determining diatom types, so other factors must be sought. The same is undoubtedly true for the Lark and the lower Tees. In the latter river the diatom communities show a well-marked difference above and below the confluence with the Skerne. This small tributary pollutes the Tees with organic matter and also increases its chloride content and its hardness. The change of communities may be due to any of these factors. If due to organic pollution then *N. viridula* is associated with sewage and *G. olivaceum* with purer water. If due to increase in hardness then *G. olivaceum* should be rare in calcareous waters and the *N. viridula* community absent in the slightly calcareous upper Tees. There are two places in the upper river which receive a marked quantity of sewage, though nothing in comparison to what flows into the lower river. These places are the Balder (see Table IV) and Barnard Castle (see Table I). At these stations both the *N. viridula* and *G. olivaceum* communities occur, but at the latter station, where numerical values are produced, the *N. viridula* is the commonest community and *G. olivaceum* considerably rarer. This suggests that the *N. viridula* community is associated with sewage contamination, and this view is confirmed by observations elsewhere. In the River Lark (see Table III) the abundance of *G. olivaceum* the first year and its scarcity the second may be accounted for by the progressive increase in organic pollution from the beet-sugar factory each year, bringing about an alteration in the composition of the water. *G. olivaceum* has been collected in quantity both in the Itchen (Hampshire) and in the Dove in Dovedale, Derbyshire, both undoubtedly pure and highly calcareous waters.

#### *Calcareous and Non-calcareous Rivers.*

In considering the macrophytic vegetation of rivers a convenient subdivision may be made on the basis of their calcium content. Insufficient collections have as yet been made of the sessile algae to decide what are the chief effects of a difference in hardness, but certain facts are indicated if one considers the Lark as highly calcareous, the lower Tees as moderately calcareous, and the upper Tees as slightly calcareous. The *Ulvella-Cocconeis* community is markedly constant in its dominant species, but it may be noted that *St. farctum* and *G. incrustans* become locally dominant in waters of high calcium content.

With the diatoms calcium content does not seem to determine the communities so much as pollution (cf. the distribution of *G. olivaceum* and *N. viridula* in the Tees). In the Lark the communities are so different from those of the Tees that it is difficult to find any basis of comparison. On the whole there seems to be a constancy of algal types in waters of

various calcium content which contrasts markedly with the changeability of the macrophytes.

*Effect of Current and Flood-water.*

A further sub-division of rivers from mountains, hills, and lowland may be made as the volume of flood-water and average speed depend to a great extent on the situation of the head-waters. The Lark and Tees may be taken as representing respectively a lowland and a mountain stream, but here again there seems to be insufficient data to make a contrast; the chief fact again seems to be the similarity of collections from very different habitats.

TABLE IV.

*Growths of Sessile Algae in Relation to the Current (average of ten collections).*

Locality.	Average speeds yds./hr.	28 days deposits mg./sq.cm.	Dominant diatoms (February to September) in order of frequency.
Below High Force.	4,210	0.6	<i>Diatoma elongatum</i> , <i>Gomphonema olivaceum</i> , <i>Achnanthes microcephala</i> , <i>Cymbella ventri- cosa</i> , <i>Nitzschia palea</i> .
Piercebridge	2,900	0.5	<i>Diatoma vulgare</i> , <i>Gomphonema olivaceum</i> , <i>Achnanthes linearis</i> , <i>Fragilaria intermedia</i> , <i>Cymbella ventricosa</i> , <i>Cocconeis placentula</i> .
Barnard Castle.	2,577	1.8	<i>Diatoma vulgare</i> , <i>D. elongatum</i> , <i>Achnanthes microcephala</i> , <i>Nitzschia palea</i> , <i>Cymbella ventricosa</i> , <i>Synedra Arcus</i> , <i>Cocconeis placent- ula</i> , <i>Navicula viridula</i> .
Above Croft.	2,475	1.0	<i>Diatoma vulgare</i> , <i>Achnanthes linearis</i> , <i>Gom- phonema olivaceum</i> , <i>Cymbella ventricosa</i> , <i>Nitzschia palea</i> , <i>N. dissipata</i> , <i>Ceratoneis Arcus</i> , <i>Navicula viridula</i> , <i>Synedra Ulna</i> , <i>Cocconeis placentula</i> .
Rokeby.	1,940	2.6	<i>Navicula viridula</i> , <i>Gomphonema olivaceum</i> , <i>Cymbella ventricosa</i> , <i>Synedra radians</i> , <i>Nitzschia palea</i> , <i>N. acicularis</i> , <i>Cocconeis placentula</i> , <i>Achnanthes microcephala</i> .
Cotherstone.	1,910	5.3	<i>Diatoma vulgare</i> , <i>D. elongatum</i> , <i>Navicula viridula</i> , <i>Cymbella ventricosa</i> , <i>Gomphonema olivaceum</i> , <i>Ceratoneis Arcus</i> , <i>Synedra Ulna</i> , <i>Cocconeis placentula</i> , <i>Nitzschia palea</i> , <i>N. dissipata</i> .

It is obvious, however, the greater the current the more difficult it is for the algae on the river-bed to retain their hold, so it is to be expected that the faster the current the sparser and less varied the growths, and these growths will consist chiefly of those genera which possess the most

efficient attaching devices. To illustrate this a number of results in the Tees are summarized in Table IV, where the commonest diatoms only are taken into consideration. In a general way these figures indicate (1) a rate of increase inversely proportional to the current, and (2) an increase in the number and variety of diatoms, and especially in the apparently non-attached forms as the current becomes slower. A fuller idea may be obtained by comparing Tables IX–XI.

## SECTION II. THE RELATION OF SESSILE ALGAE TO POTAMOPLANKTON.

Having therefore established the presence of these sessile growths on the river-bed, the next matter to examine is how far they contribute to the plankton of rivers. In this connexion one can only profitably consider those species with a definite distribution and a limited habitat. The origin of algae such as *Oscillatoria* and *Pediastrum*, known to flourish in a variety of habitats, must remain in doubt when found in a river, but nearly all algal communities possess characteristic species which greatly assist in the elucidation of the problems of origin.

### *Potamoplankton.*

This term was established by Zacharias (42) to designate the special community of free-floating micro-organisms found in a river. According to him the nature of the potamoplankton is determined by that of the pools and backwaters associated with the river system as well as by the strength of the current. But as Purdy (35) has pointed out, the current of a river washes all unattached organisms towards the sea where they are destroyed. This applies to all floating organisms, both those recognized as purely adventive (e.g. *Gomphonema* spp.) as well as those usually considered to be truly planktonic and occasionally found in rivers (*Anabaena*, *Pandorina*, &c.). There must, therefore, be some source or sources from which the organisms composing the potamoplankton are continuously replenished. According to Krieger (23) these sources from which the plankton of a river originates are :

1. The districts adjoining the source.
2. The Heleoplankton (i.e. from pools on the system).
3. The Limnoplankton (i.e. from lakes on the system).
4. Drains and tributaries.

To these must now be added a fifth, and in some cases a very important source, namely, the algal vegetation on the river-bed. This latter ceases to be of importance only in large, deep rivers where the volume of water is great. Here the area of the river-bed, submerged banks and macrophytes,

sufficiently well illuminated to support algal growth, is small. Rivers where such conditions exist are rare in England, but many of those studied by other workers (e.g. the Illinois, Rhine, and Danube) are of the deep kind, and they contain an altogether different potamoplankton from that recorded in the smaller rivers.

#### IMPORTANCE OF THE VARIOUS SOURCES OF RIVER PLANKTON.

##### 1. The River-bed.

In all the English rivers investigated, namely, the Itchen, Lark, Tees, Tern, Cam, and Nene, as previously mentioned, large growths of algae have been found on the river-bed. The continual movement of the current over these growths will wash away a certain number of individuals or portions of individuals which will float downstream to the sea unless they are caught up against some obstacles. These sessile algae are thus an important source of supply for the river plankton, and in small streams probably a far more important source than any of those mentioned by Krieger (l.c.).

The quantity of sessile algae in the plankton will depend on :

1. The extent of the growth on the river-bed.
2. The rate of flow of the water above the growth.
3. The nature of the means of attachment of the various species making up the growth.
4. The buoyancy of the growth.

In the first place, given a portion of the river-bed with a constant current flowing over it, the number of algae washed away from the sessile growths will be proportional to the rate of the current and volume of the growth. But if the current be increased, as in times of flood, the augmented swirl will wash off a greater number in the same time—perhaps even all of them. Though often the case, there will not necessarily be an increase in the proportion of plankton since the volume of water is also increased. The point of importance lies in the *difference* between mean and augmented currents. Station A has a slow normal current and Station B has a fast normal current. Under ordinary conditions less will probably be washed away from B with its fast current and therefore sparser and more securely attached growths, than at A with a much denser growth of insecurely attached plants. When the current is increased at A, even if it remains less than that normally occurring at B, a greater number of algae will be washed off than usual and the number will also exceed the number normally washed off at B in spite of the current at B being still faster.

*Growth-types of Sessile Algae.*

One can distinguish from the frame results six types of algal growths based on their method of attachment.

(a) The 'thalloid' type comprises those algae that are closely appressed and firmly attached by mucilage or other means along a large part of their surface. They are multicellular or colonial forms, e.g. *Ulvella*, *Stigeoclonium farctum*, *Oncobrysa*.

(b) The 'Cocconeis' type comprises those diatoms, that are attached to the substratum by the whole of one surface, e.g. *Cocconeis* and possibly some species of *Amphora* and *Cymbella*.

(c) The 'filamentous' type comprises those filamentous algae that are attached to the substratum by a holdfast, e.g. *Ulothrix* or by a mucilaginous film, e.g. *Phormidium*.

(d) The 'stalked' diatom type includes many genera of diatoms all of which are loosely attached to the substratum by a branched or unbranched mucilaginous pedicel (e.g. *Gomphonema*), a mucilaginous tube (e.g. *Cymbella* § *Encyonema*) or by a mass of mucilage at one end, e.g. *Synedra* and *Diatoma*.

(e) The 'unattached' type comprises a tremendous number of colonial Chlorophyceae and Myxophyceae, desmids and diatoms that have no obvious method of attachment, e.g. *Cyclotella*, *Scenedesmus*, *Closterium*.

(f) The 'motile' type includes all those algae that are obviously adapted to a free-swimming existence because they possess cilia or flagellae.

Of these six groups the thalloid type, though abundant on the river-bed, is not represented in the plankton except as fragments or portions such as projecting threads of *S. farctum*. The *Cocconeis* type only differs from the thalloid type in that when growths are detached from the substratum it is as a complete individual and not as fragments, and they can thus be recognized when found in the plankton. Diatoms of this type are frequently represented in the plankton, but not in proportion to their abundance on the river-bed compared to the stalked and non-attached diatoms. These two types of growth above include almost the whole of the *Ulvella-Cocconeis* community.

The stalked diatom type will be abundantly represented in the plankton more or less in proportion to their numbers on the river-bed at the time, and inversely as the strength of the attaching devices. They will probably be most frequent after floods and in the shallow and more rapidly flowing reaches. Also there will probably be a selective action based on the size of the organisms and the nature of the aggregate growths. Large and freely divergent algae like *Synedra* and *Meridion* will be more frequently detached than the very small and aggregated species like *Achnanthes*.

A very similar type in these respects is the filamentous type, as here again algae of this type are attached only by a small portion of the plant. Because of the nature of the filaments, fragmentary portions rather than complete plants are seen in the plankton, and more rarely so than the diatoms, because the strength of a filament is obviously greater than the strength of a mucilaginous stalk. Usually they are found in great abundance when growths in the river-bed are dying and breaking up. These last two types are very well represented, both in the sessile and free-floating diatom communities of rapidly flowing water, e.g. the *Diatoma-Gomphonema-Achnanthes* community of the swifter part of the Tees (see Table IX). They are also represented in slower rivers, e.g. the *Synedra Ulna* community of the Lark, but mixed with other types, especially with the unattached diatom type.

In relation to the unattached type (*e*) which includes *Cyclotella*, *Navicula*, *Scenedesmus*, and *Pediastrum* a certain difficulty arises. The algae included here have no visible means of attachment to indicate an epiphytic habit, and they have been frequently collected in ponds among the free-floating algae. Yet this is no proof of their normal habit unless at the same time the littoral zone and the mud have also been examined. Haphazard collections made in all types of streams and ponds show that a tremendous variety of species of this group do occur on mud, on stones, or on submerged macrophytes in sufficient quantity to form brown films or flocks. A list of these is given in Table V. Usually the method adopted for collecting has been to take either some of the mud or the macrophytic vegetation and smear it over a slide.

As this group of organisms can therefore occur in such a variety of habitat as on mud, in pond and slow stream, on macrophytic vegetation, in the littoral zone, freely floating in ponds or rivers, it is obvious when found on the river-bed with epiphytic algae of growth types (*a*) to (*d*) they may just as well be normal bottom forms as plankton forms caught up among the growths on the river-bed. Types (*a*) to (*d*) are usually considered epiphytic, and it is misleading to give the same name to those algae of the non-attached type of growth (Type *e*) which extensive collection has shown to be very prevalent on the bottom of stream, pond, or ditch. As these algae occur in such a variety of habitat they will throughout this paper be referred to as 'cosmopolitan' forms. Examples of this type may be found in nearly all communities, but their number and importance become greater the slower the stream. In the sessile communities they are represented by *S. obliquus* in the *Ulvella-Cocconeis* community and by *N. radiosa*, *N. viridula*, *S. ovata* in the diatom communities of the slower-flowing portions of rivers. In the free-floating communities they are also abundantly represented by *Cyclotella*, *Pediastrum*, and *Scenedesmus* spp. Algae of this type will obviously be more easily washed up from the bottom than

TABLE V.

Summary of Collections from a Variety of Habitat.

- (a) *River Snail at Fordham, Cambridgeshire, June 1927.*  
A small calcareous stream six feet wide. Current little. Collection from mud in middle of slow reach.  
*Cymatopleura Solea*, *Nitzschia sigmoidea*, *Navicula viridula*, *Gomphonema olivaceum*.
- (b) *Swaffham Lode at Swaffham Bulbeck, Cambridgeshire, April 1927.*  
A small drainage dyke six feet wide. Current almost none. Collection from mud at the side.  
*Synedra capitata*, *Cymbella aspera*, *Nitzschia acicularis*, *Achnanthes Hungarica*, *Cyclotella comta*, *Diatoma elongatum*.
- (c) *Drainage Dyke in Chippenham Fen, Cambridgeshire, March 1926.*  
A small fenland drainage dyke four feet wide filled with *Phragmites*. No current. Collection from the stems of *Phragmites*.  
*Melosira varians*, *Meridion circulare*, *Synedra Ulna*, *Fragilaria capucina*.
- (d) *Stream between Trumpington and Cherry Hinton, Cambridge,<sup>1</sup> Sept. 1927.*  
A small calcareous stream four feet wide. Current fast. Collection made from stones.  
*Cocconeis placentula*, *Gomphonema constrictum*, *G. olivaceum*, *Gyrosigma attenuatum*, *Navicula lanceolatum*, *Rhoicosphenia curvata*.
- (e) *Ditch at Hertford Heath, January 1926.*  
A small ditch with water from a pond probably dry in summer. Current moderate. Collection from mud on the bottom which was thick with a brown growth.  
*Gyrosigma attenuatum*, *Navicula lanceolata*, *Nitzschia sigmoidea*, *Achnanthes Hungarica*.
- (f) *River Welland at Fosdyke, February 1926.*  
A small river with very little current and slight tidal movement. Collection from *Myriophyllum* in a shallow part.  
*Synedra pulchella*, *Surirella ovalis*, *Rhoicosphenia curvata*, *Navicula cryptocephala*.
- (g) *River Anker at Tamworth, April 1926.*  
A small river with moderate current. Collection of weeds, mud, and debris by the bank just below a sewage outfall.  
*Navicula viridula*, *N. Reinhartii*, *Synedra Ulna*, *Nitzschia palea*, *Melosira varians*, *Amphora ovalis* var. *minutissima*.
- (h) *River Trent, below Colwick, Notts., February 1926.*  
A large river with moderate current. Collection from mud and stones near the bank.  
*Navicula lanceolata*, *Synedra Ulna*, *Cyclotella Meneghiniana*, *Surirella ovalis*.
- (i) *Experimental Fish-pond, Alresford, Hants., September 1925.*  
A small concrete pond fed by River Itchen. Collection from slate left in middle of pond for a fortnight.  
*Amphora ovalis*, *Navicula Reinhartii*, *Cocconeis placentula*, *Gyrosigma attenuatum*, *Achnanthes Hungarica*, *Nitzschia dissipata*, *Cymatopleura Solea*.
- (j) *Old Alresford Pond, Hants., September 1925.*  
A large pool connected to River Alre. Collections taken from among the littoral vegetation of *Glyceria aquatica*.  
*Eunotia lunaris*, *Nitzschia palea*, *Gomphonema acuminatum*, *Achnanthes Hungarica*, *Navicula viridula*, *N. pseudo-bacillum*.

<sup>1</sup> I am indebted to Mr. R. H. Mobbs for this list.

those of the other types (*a-d*), and in considering the origin of such algae each species has to be taken in relation to its known distribution and quantity. Algae of the motile type of growth comprise a free-floating community which is best represented in the larger continental rivers though also present in the Thames (Fritsch (13)). Algae of this type are clearly only found on the river-bed as casual individuals caught up, though *Euglena* spp. have been collected as dominant growths on the mud near a sewage outfall with a considerable current over the surface.

It has been pointed out by Butcher, Pentelow, and Woodley (10) that in the River Lark diatoms are responsible for the production of large quantities of oxygen which leads to super-saturation. At the same time bubbles of gas appear on the surface of growths on the river-bed, increasing their buoyancy so that masses rise to the surface and float away downstream. The same phenomenon is familiar in ponds, showing that here also there are abundant bottom growths. In streams it constitutes a factor tending to introduce these sessile algae into the plankton which effect will be greatest when photosynthesis is greatest. Given that the growths on the river-bed are the only source of the plankton the latter should show the following characteristics :

1. It should present the same composition as the sessile growths, save that it will only very rarely contain algae of growth type (*a*).
2. The effect of a flood will be to increase the diversity of the plankton by introducing certain sessile forms rarely seen at other times.
3. Violent fluctuations will be observed in sunny weather owing to the rising from the bottom of buoyant growths from the river-bed.
4. There will be a total absence of free-swimming forms such as *Volvox*, *Pandorina*, *Ceratium*.

### 2. *The Districts Adjoining the Source.*

Krieger (l.c.) himself states that the sources of a river are without plankton and that the algae in the bogs and marshes around are soon destroyed after being washed into the river. There seems to be no essential difference between the bogs and marshes at the source and the bogs and marshes lower down in the river system, the algae of which would be washed into the river either by way of ponds, tributaries, or drains. In any case, the algae of such habitats can be said to be benthic rather than planktonic in origin.

### 3. *The Limnoplankton.*

The method of supply postulates the occurrence of a lake-like enlargement in the course of the river, and this is exceptional in Britain. In any case limnoplankton contains characteristic organisms which are easily



recognized as derived from such a source when found in a river. An example is furnished by *Gonatozygon monotaenium* recorded from the Wharfe, which probably came from the reservoirs in a subsidiary valley (the Washburn). If potamoplankton were, in the main, recruited from limnoplankton many of the rivers of this country would be barren. When a river stands in connexion with a lake, the limnoplankton adds to the variety of the potamoplankton, but there is no evidence that the organisms of the former find favourable conditions in the river, since they exhibit no marked increase in numbers as they pass downstream.

#### 4. *The Heleoplankton.*

Most rivers include within their watershed pools and large dykes, the heleoplankton of which will contribute to the variety of the potamoplankton. The same may be said of the heleoplankton as of the limnoplankton, namely, that it is often composed of characteristic species whose origin is readily recognized; that there is a plankton in rivers with no such source of supply (e.g. the upper Tees and Itchen) and that there is little evidence that the organisms of the heleoplankton, even if they reach the river, multiply there to any extent.

#### 5. *Tributaries and Drains.*

Like the main river, tributaries must have a source from which their plankton is recruited, and thus in considering them we are only extending inquiry higher up the watercourse. The algae of ditches (as well as any other place) are either epiphytic (e.g. *Synedra Ulna* and *Gomphonema* spp.) living in the shelter of weeds or on the bottom (e.g. *Gyrosigma*, *Nitzschia*) or in the open water, e.g. *Volvox*. The epiphytic forms and those unattached or on the bottom may be identical to those on the river-bed (especially if the river is small and shallow) and are brought into the river plankton under exactly similar conditions as those derived from the river-bed; the free-floating forms are really in the nature of heleoplankton. Such ditches bear a close resemblance to the quieter channels of rivers, to backwaters, and the like, and one may therefore expect algae from such habitats to exist longer in the river than those derived from lakes and pools. A large number of algae of all descriptions flourish in ditches and drains, but there are no characteristic free-swimming organisms which unmistakably indicate their origin, and the common *Volvocales* and *Chlorococcales* found there are known in such a variety of habitats, namely, in marshes, pools, water-butts, among littoral macrophytes, or even on the bottom of pond or river, that it is difficult to decide their origin or whether they have existed long in the river when met with in its plankton.

Algae from drains and ditches will find their way into the river only when the former are overflowing. This may be the case almost

continuously, or it may only occur spasmodically at times of heavy rainfall or when adjustments are made to the sluices. Each case must therefore be studied on its merits. Fritsch (14) examined the plankton of several backwaters of the Thames, and his lists show a greater quantitative development and less variety (especially in the motile community) than in the main river, but the collections show such a difference, markedly so in the rarity of *Volvocales* in the main river, that his collections cannot be considered as proving that backwaters are an important source of potamoplankton.

From these considerations it is evident that on examining the plankton of any river it is possible to refer certain constituent organisms to their source of origin, namely, limnoplanktons like *Micrasterias* and *Gonatozygon* to lakes, heleoplanktons like *Volvox* and *Ceratium* to pools, epiphytic algae like *Cocconeis* and *Synedra Ulna* to the river-bed or the weeds. But there are a number of algae found in a stream whose source of origin is doubtful. These belong to growth Type (e) above (p. 833), and their quantity apparently increases in large streams and rivers. There is some evidence produced that several may be considered bottom-living forms in spite of the absence of any obviously attaching organs, but a great deal of additional data is required to establish their normal habitat.

#### GENERAL CONSIDERATION OF THE AVAILABLE DATA WITH REFERENCE TO POTAMOPLANKTON AND ITS SOURCES OF SUPPLY.

##### I. *Rivers of Great Britain.*

Lists of potamoplankton are available from the following British rivers:

- The Thames, Fritsch (13, 14).
- „ Cam, Fritsch (15).
- „ Trent, Fritsch (15).
- „ Wharfe, Butcher (8) and Schroeder (38).
- „ Shannon, Southern and Gardiner (39).
- „ Lark, Butcher, Pentelow, and Woodley (11).

Details of the algae on the river-bed are only available from the Lark, but to this published data can now be added the results of twelve months' investigations on the microflora of the River Tees.

##### (a) *The River Tees.*

The Tees has very few tributaries of any size, and also there are no lakes in the river basin from which limnoplankton could be washed into the river. Because of its swiftness also, there are no backwaters and there is very little chance of reproduction in the stream itself as it normally takes the water thirty hours to flow from High Force to tidal limit, a distance of sixty miles. There are, however, two reservoirs situated on the tributaries

Balder and Lune respectively, but the list (Table VI) of the algae collected by centrifuge from the water of the Balder within twenty yards of its junction with the Tees, does not include any characteristic members of the heleoplankton and limnoplankton communities.

TABLE VI.

*Free-floating Organisms from the River Balder.*

*Taken monthly at 'Balder Foot' from June 1929–June 1930.*

Arranged in order of frequency.

<i>Navicula viridula</i>	.	.	.	} In all twelve collections. Abundant in six.
<i>Synedra Ulna</i>	.	.	.	
<i>Diatoma vulgare</i>	.	.	.	
<i>Diatoma elongatum</i>	.	.	.	
<i>Cocconeis placentula</i>	.	.	.	In ten collections.
<i>Cymbella ventricosa</i>	.	.	.	} In nine collections.
<i>Ceratoneis Arcus</i>	.	.	.	
<i>Nitzschia acicularis</i>	.	.	.	} In eight collections.
<i>Nitzschia palea</i>	.	.	.	
<i>Synedra Arcus</i>	.	.	.	
<i>Synedra tenera</i>	.	.	.	
<i>Gomphonema olivaceum</i>	.	.	.	
<i>Oscillatoria brevis</i>	.	.	.	In six collections.
<i>Scenedesmus obliquus</i>	.	.	.	} In five collections.
<i>Scenedesmus quadricauda</i>	.	.	.	
<i>Achnanthes linearis</i>	.	.	.	} Seen in three collections.
<i>Achnanthes microcephala</i>	.	.	.	
<i>Tabellaria flocculosa</i>	.	.	.	
<i>Cymbella pusilla</i>	.	.	.	
<i>Asterionella formosa</i>	.	.	.	
<i>Staurastrum bienianum</i>	.	.	.	
<i>Meridion circulara</i>	.	.	.	} Seen in one collection.
<i>Melosira varians</i>	.	.	.	
<i>Pinnularia viridis</i>	.	.	.	
<i>Ulothrix subtilissima</i>	.	.	.	
<i>Fragilaria virescens</i>	.	.	.	
<i>Closterium Leibleinii</i>	.	.	.	
<i>Ankistrodesmus falcatus</i>	.	.	.	

Collections have been made at fifteen stations on the Tees, and details are given for the same four stations as for the sessile algae. Table VII shows the chief members of the free-floating microflora and, as in the case of the sessile microflora, certain communities can be indicated.

In the first place there is no equivalent of the *Ulvella-Cocconeis* community. *Cocconeis* does occur in the summer months but is rarely dominant, and this is doubtless due not to its scarcity but to the strength of its mode of attachment as compared to such diatoms as *G. olivaceum* and *N. viridula*.

TABLE VII.  
River Tees. Common Species (more than 10 per c.c.) in the Free-floating Microflora. June 1929 to May 1930.

Date.	Yarm.	Eryholme.	Barnard Castle.	High Force.
June 19	<i>Navicula viridula</i> (16)	<i>Cymbella ventricosa</i> (118) <i>Navicula viridula</i> (53) <i>Diatoma vulgare</i> (33) " <i>elongatum</i> (28) <i>Cyclotella Meneghiniana</i> (23) <i>Cocconeis placentula</i> (23)	<i>Cymbella ventricosa</i> (116) <i>Cocconeis placentula</i> (27) <i>Nitzschia palea</i> (17) <i>Cymbella pusilla</i> (12)	<i>Cymbella ventricosa</i> <i>Cocconeis placentula</i>
July 17	<i>Cyclotella Meneghiniana</i> (200) <i>Scenedesmus quadricauda</i> (200) " <i>obliquus</i> (164) <i>Cymbella ventricosa</i> (72) <i>Ankistrodesmus falcatus</i> (40) <i>Scenedesmus biungatus</i> (34) <i>Nitzschia acicularis</i> (33) <i>Synedra tenera</i> (32) <i>Cosmarium Turpinii</i> (26) <i>Nitzschia palea</i> (25) " <i>signata</i> (20) <i>Cocconeis placentula</i> (12)	<i>Cocconeis placentula</i> (110) <i>Achnanthes linearis</i> (75) <i>Nitzschia palea</i> (33) <i>Cyclotella Meneghiniana</i> (20) <i>Ankistrodesmus falcatus</i> (13)	<i>Nitzschia palea</i> (25) <i>Cocconeis placentula</i> (22) <i>Cymbella ventricosa</i> (18)	<i>Asterionella formosa</i> (28) <i>Cocconeis placentula</i> (19) <i>Nitzschia palea</i> (14)
August	<i>Cyclotella Meneghiniana</i> (28)	<i>Cyclotella Meneghiniana</i> (106) <i>Nitzschia palea</i> (124) <i>Navicula viridula</i> (56) " <i>cryptocephala</i> (46) <i>Nitzschia dissipata</i> (44) <i>Navicula rhyncephala</i> (39) <i>Cocconeis placentula</i> (32) <i>Scenedesmus quadricauda</i> (14) <i>Cymbella ventricosa</i> (11)	<i>Nitzschia palea</i> (149) " <i>acicularis</i> (28) <i>Synedra Ulma</i> (28) <i>Nitzschia dissipata</i> (21) <i>Cocconeis placentula</i> (14)	<i>Navicula viridula</i> (16) <i>Nitzschia palea</i> (11)

September	<i>Cyclotella Meneghiniana</i> (78)	<i>Cyclotella Meneghiniana</i> (86)	<i>Nitzschia palea</i> (91) " <i>acicularis</i> (22) <i>Cyclotella Meneghiniana</i> (15)	<i>Nitzschia palea</i> (35)
October	<i>Cocconeis placentula</i> (40) <i>Navicula viridula</i> (48) " <i>cryptoccephala</i> (46) <i>Cyclotella Meneghiniana</i> (21) <i>Nitzschia palea</i> (11)	<i>Nitzschia palea</i> (19) <i>Cocconeis placentula</i> (12)	—	—
November	—	—	—	—
December	—	—	—	—
January	—	<i>Sphaerotilus natans</i> (25)	—	—
February	—	<i>Navicula viridula</i> (12)	—	—
March	—	<i>Sphaerotilus natans</i> (47) <i>Navicula viridula</i> (19)	—	<i>Diatoma vulgare</i> (30) " <i>elongatum</i> (13)
April	<i>Navicula viridula</i> (44) <i>Gomphonema olivaceum</i> (12)	<i>Navicula viridula</i> (63) <i>Gomphonema olivaceum</i> (20) <i>Cymbella ventricosa</i> (19) <i>Nitzschia palea</i> (18) <i>Cocconeis placentula</i> (12) <i>Nitzschia dissipata</i> (16)	<i>Nitzschia palea</i> (22) <i>Gomphonema olivaceum</i> (18) <i>Sphaerotilus natans</i> (17)	<i>Gomphonema olivaceum</i> (19)
May	<i>Navicula viridula</i> (72) <i>Nitzschia acicularis</i> (63) <i>Cymbella ventricosa</i> (43) <i>Nitzschia palea</i> (23) <i>Gomphonema olivaceum</i> (35) <i>Synedra Acus</i> (22) <i>Diatoma vulgare</i> (14) " <i>elongatum</i> (11)	<i>Sphaerotilus natans</i> (120) <i>Navicula viridula</i> (61) <i>Cymbella ventricosa</i> (40) <i>Gomphonema olivaceum</i> (11)	<i>Cymbella ventricosa</i> (58) <i>Nitzschia palea</i> (23) <i>Synedra Acus</i> (24) <i>Gomphonema olivaceum</i> (17) <i>Navicula viridula</i> (15) <i>Achnanthes linearis</i> (12) <i>Diatoma vulgare</i> (12)	<i>Diatoma elongatum</i> (28) <i>Gomphonema olivaceum</i> (22) <i>Achnanthes microcephala</i> (13)

The diatom communities are as varied as in the sessile flora. At High Force the chief communities are *Diatoma-G. olivaceum* in the spring and *N. palea* in the summer. The first corresponds to the sessile communities at the same time (see Table IX). The only organism not clearly originating from the sessile growths is *A. formosa*, and this must have been derived from some bog-pool or quiet stretch of the river. Nor was this diatom present in other collections made in the same day from four places lower down the river, and this would seem to imply that it failed to survive.

At Barnard Castle the same communities seem to be present with the addition of *A. ovalis* f. *minutissima* in part replacing the *Diatoma-Gomphonema* community.

At Eryholme, as with the sessile, so with the free-floating communities there is a marked change. A community with *N. radiosa* is the most marked and other species in order of frequency of occurrence are *Cymbella ventricosa*, *N. palea*, and *G. olivaceum*. Again, all these bear a marked relationship to the sessile communities, though there is a greater intermingling of the species, which is to be expected, as the flowing water passes over the sessile growths and picks up algae throughout its course from source to mouth. At Eryholme there is, however, a community which also is more definitely developed at Yarm in the summer, and in which *C. Meneghiniana* is the dominant form, and associated with it are *Scenedesmus* spp. and *A. falcatus*. This community does not show the definite relationship to the sessile growths that the other communities do and therefore may be due to influences other than the river-bed. On the other hand, such forms as *Cyclotella* do occur in quantity among the sessile growths but very spasmodically. It is difficult at the moment to decide the source of origin of this community—it is seasonal in its appearance, its constituents are erratic, and it occurs in a fast-flowing water where backwaters and similar breeding grounds are extremely few.

The distribution of these communities is brought out in Table VII a which shows (a) in summer and autumn: *C. Meneghiniana* dominant at Yarm, *N. palea* dominant in the upper reaches, an intermingling of the two communities at Eryholme, and *Cyclotella* occurring rarely in the upper reaches; (b) in the spring: *D. vulgare* and *G. olivaceum* dominant at High Force, *N. radiosa* dominant at Eryholme and Yarm, with an intermingling of communities both at Eryholme and Barnard Castle.

It was thought that communities similar to the *Cyclotella* community with characteristic plankton organisms (e.g. *Pandorina*) might occur in the tidal rise and fall of fresh water between Yarm and Stockton, so collections were made every mile during the high tide on June 18, 1930, both with the tow-net and the centrifuge. The results (Table VIII) show that all the ten samples are very similar to one another and to those from the upper

river, and there is no increase of any organism that might be considered as euplanktonic.

TABLE VII a.

*Dominant Species of Free-floating Communities in nos./c.c.*

Station.	Species.	Aug.	Sept.	Oct.
High Force	<i>Nitzschia palea</i>	11	35	5
	<i>Cyclotella Meneghiniana</i>	—	6	—
Barnard Castle	<i>Nitzschia palea</i>	149	91	5
	<i>Cyclotella Meneghiniana</i>	—	15	—
Eryholme	<i>Nitzschia palea</i>	125	7	20
	<i>Cyclotella Meneghiniana</i>	106	86	2
Yarm	<i>Nitzschia palea</i>	3	2	22
	<i>Cyclotella Meneghiniana</i>	28	78	6
		Mar.	Apr.	May.
High Force	<i>Diatoma</i> spp.	43	11	30
	<i>Gomphonema olivaceum</i>	4	20	22
	<i>Nitzschia palea</i>	1	3	9
	<i>Cymbella ventricosa</i>	—	—	2
	<i>Navicula viridula</i>	—	—	—
Barnard Castle	<i>Diatoma</i> spp.	4	18	12
	<i>Gomphonema olivaceum</i>	8	18	17
	<i>Nitzschia palea</i>	2	22	23
	<i>Cymbella ventricosa</i>	1	4	58
	<i>Navicula viridula</i>	6	19	15
Eryholme	<i>Diatoma</i> spp.	4	8	8
	<i>Gomphonema olivaceum</i>	2	21	11
	<i>Nitzschia palea</i>	10	18	60
	<i>Cymbella ventricosa</i>	1	37	40
	<i>Navicula viridula</i>	19	188	61
Yarm	<i>Diatoma</i> spp.	1	8	25
	<i>Gomphonema olivaceum</i>	5	12	35
	<i>Nitzschia palea</i>	4	6	23
	<i>Cymbella ventricosa</i>	1	9	43
	<i>Navicula viridula</i>	9	44	72

An attempt is made in Tables IX, X, and XI to compare the actual numbers of the free-floating and sessile algae (estimated on slides) at three stations, but it is difficult to decide the basis of comparison between an area of river-bed and a given volume of water. The river, except at Yarm, is on an average 100 cm. deep. If the whole of the suspended organisms, uniformly distributed in the water, were deposited on the bed, each sq. cm. of the latter would receive the number of organisms in a cylinder with a sectional area of 1 sq. cm. and a height equal to the depth of the water. As the latter is taken as 100 cm., the numbers of free-floating algae are given per 100 c.c. The conclusions that can be based on these tables are:

1. There are a certain number of organisms attached along the whole of one flat surface that are never represented in the plankton, e.g. *Ulvella*.

2. The rest of the algae are represented in both communities with a few exceptions.

3. The filamentous algae (e.g. *Ulothrix* and *Oedogonium*) although at times abundant in the sessile growth are rare in the plankton.

TABLE VIII.

*Free-floating Microflora from below Yarm. June 5, 1930.*

	Yarm.											Stockton Bridge.
Miles below Yarm.		1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	
<i>Cyclotella Meneghiniana</i> .	c	r	r	rr	rrr	rrr	rr	—	rr	—	—	
<i>Diatoma elongatum</i> .	ccc	—	—	—	c	r	c	c	c	r	rr	
" <i>vulgare</i> .	ccc	cc	c	c	cc	cc	r	rr	cc	c	r	
<i>Synedra Ulna</i> .	r	c	rrr	r	cc	cc	ccc	ccc	cc	cc	r	
<i>Ceratoneis Arcus</i> .	r	—	rrr	rrr	—	rrr	—	—	rrr	rr	rr	
<i>Achnanthes exilis</i> .	r	c	c	c	rr	rrr	r	c	c	c	r	
" <i>linearis</i> .	c	c	c	c	c	c	c	c	cc	cc	rr	
<i>Cocconeis placentula</i> .	c	—	rrr	rr	—	c	c	c	r	rr	r	
<i>Navicula viridula</i> .	ccc	ccc	ccc	ccc	ccc	ccc	ccc	ccc	ccc	ccc	ccc	
" <i>cryptocephala</i> .	c	—	—	—	r	c	cc	cc	c	c	r	
" <i>gracilis</i> .	c	rrr	rrr	rrr	rrr	—	—	—	rrr	—	—	
<i>Gomphonema olivaceum</i> .	cc	c	cc	c	c	cc	c	c	cc	cc	c	
<i>Cymbella delicatula</i> .	rr	—	rrr	—	—	—	—	—	rrr	—	—	
" <i>ventricosa</i> .	ccc	ccc	ccc	ccc	ccc	ccc	ccc	ccc	ccc	ccc	ccc	
<i>Surirella ovata</i> .	c	c	cc	cc	c	cc	ccc	cc	cc	ccc	cc	
<i>Nitzschia acicularis</i> .	ccc	cc	ccc	ccc	cc	cc	ccc	ccc	ccc	cc	c	
" <i>palea</i> .	ccc	—	r	r	rr	—	—	rr	r	rr	rrr	
" <i>dissipata</i> .	r	—	—	—	—	—	—	—	—	—	—	
<i>Oscillatoria brevis</i> .	—	rrr	—	—	—	—	—	—	—	—	—	

ccc = abundant.

cc = common.

c = uncommon.

r. = rare.

rr = very rare.

rrr = single individual seen.

4. Even on the basis of comparison above mentioned the numbers of the sessile algae usually greatly exceed the number of the plankton algae.

5. The free-floating communities bear an obvious relation both in character and the numbers present to the sessile communities. This is well marked in most cases, but very erratic in certain algae with no means of attachment and which fall chiefly into a *Cyclotella-Scenedesmus* community.

6. The *Cyclotella* community consists of algae with no attachments. They are often found on the river-bed as well as in the water and at places other than below Croft, although here they reach their best development. The origin of this community is still obscure, though in the case of other diatoms with no attachments, e.g. *N. viridula* and *S. ovata*, there seems to be sufficient evidence to show that they multiply abundantly on the river-bed.



TABLE IX.  
Comparison of Sessile and Free-floating Communities. River Tocs at High Force.

Date of collecting—	24. 2. 30		24. 3. 30		23. 4. 30		22. 5. 30	
	1,150 Fr.-fl.	Sess. 5,700	5,640 Fr.-fl.	Sess. 169,600	5,270 Fr.-fl.	Sess. 7,040	10,750 Fr.-fl.	Sess. 130,000
No. of free-floating algae/100 c.c. " " sessile algae/sq. cm.								
<i>Meridion circulare</i> . . . . .	—	70	40	—	90	460	100	170
<i>Diatoma vulgare</i> . . . . .	50	200	2,960	5,300	170	260	160	—
" <i>elongatum</i> . . . . .	200	160	1,330	29,000	920	1,720	2,840	77,500
<i>Synedra Ulna</i> . . . . .	—	+	—	—	40	50	180	350
" <i>acis</i> . . . . .	100	20	—	—	—	20	—	—
" <i>tenera</i> . . . . .	—	100	—	5,700	—	310	340	1,250
<i>Ceratonopsis Arcus</i> . . . . .	10	80	40	1,000	240	720	880	1,250
<i>Achnanthes microcephala</i> . . . . .	80	3,700	60	53,000	220	250	1,340	98,500
" <i>linearis</i> . . . . .	150	—	40	1,900	340	70	—	—
" <i>exilis</i> . . . . .	—	—	20	—	200	—	870	400
<i>Cocconeis placentula</i> . . . . .	40	—	—	—	100	—	—	—
<i>Navicula cryptocephala</i> . . . . .	30	—	60	—	270	10	—	—
<i>Gomphonema constrictum</i> . . . . .	—	20	30	—	—	—	—	—
" <i>parvulum</i> . . . . .	—	100	—	2,300	90	120	—	—
" <i>olivaceum</i> . . . . .	—	370	370	15,500	1,950	2,800	2,240	4,100
<i>Cymbella delicatula</i> . . . . .	290	100	40	900	—	—	310	250
" <i>ventricosa</i> . . . . .	60	—	—	—	—	10	230	250
<i>Nitzschia palea</i> . . . . .	—	—	—	—	—	80	850	700
" <i>dissipata</i> . . . . .	30	—	—	—	70	20	+	+
Other diatoms . . . . .	+	+	30	100	+	+	60	1,000
<i>Ulothrix zonata</i> . . . . .	—	—	—	—	—	—	140	—
" <i>subtilissima</i> . . . . .	—	580	—	39,800	—	—	50	8,000
<i>Oscillatoria brevis</i> . . . . .	—	—	—	—	—	10	—	—
<i>Ulvella frequens</i> . . . . .	—	—	—	1,600	—	—	—	800
<i>Chaetophelis megalocystis</i> . . . . .	—	—	—	12,400	—	—	—	—

+ = only one individual seen. Dominant organisms are shown in heavy figures.

TABLE X.

Comparison of Sessile and Free-floating Communities—River Tees at Eryholme.

Date of collecting—	15. 7. 29		19. 8. 29		16. 9. 29		22. 10. 29	
No./sq. cm. of sessile algae	Fr.-fl.	90,900	Fr.-fl.	63,100	Fr.-fl.	44,300	Fr.-fl.	114,600
No./100 c.c. of free-floating algae	27,760	Sess.	54,000	Sess.	11,450	Sess.	5,790	Sess.
<i>Cyclotella Meneghiniana</i>	2,000	1,000	10,460	300	8,570	800	170	100
<i>Meridion circulare</i>	—	—	—	—	—	—	—	—
<i>Diatoma vulgare</i>	80	—	—	—	270	—	200	10
„ <i>elongatum</i>	—	—	—	—	—	—	—	—
<i>Synedra Ulna</i>	120	400	30	—	310	600	880	3,200
„ <i>Acutus</i>	640	—	—	—	—	—	—	—
„ <i>tenera</i>	—	—	—	—	—	—	—	—
<i>Ceratoneis Arcus</i>	—	800	—	—	—	—	—	—
<i>Achnanthes exilis</i>	—	—	—	—	—	—	—	—
„ <i>linearis</i>	7,520	—	—	—	—	—	—	7,700
„ <i>microcephala</i>	—	500	1,050	—	—	—	120	—
<i>Cocconeis placentula</i>	10,960	19,400	3,190	8,800	520	28,700	1,270	3,200
<i>Navicula viridula</i>	—	—	5,620	100	40	300	180	—
„ <i>cryptcephala</i>	200	—	4,600	—	—	—	80	—
„ <i>rhynchocephala</i>	—	—	3,960	—	—	—	—	400
<i>Gomphenema constrictum</i>	—	—	—	—	—	600	—	—
„ <i>olitoaceum</i>	160	—	—	—	—	—	90	—
<i>Cymbella delicatula</i>	—	—	—	—	—	—	30	—
„ <i>ventricosa</i>	—	2,100	1,120	100	70	300	280	200
<i>Nitzschia palea</i>	3,320	1,300	12,390	—	640	400	1,950	200
„ <i>acicularis</i>	—	—	3,920	—	280	200	210	—
„ <i>dissipata</i>	—	—	4,420	—	160	—	120	—
„ <i>signa</i>	—	—	280	—	—	—	—	—
<i>Surirella ovata</i>	—	—	—	—	—	—	—	—
<i>Scenedesmus obliquus</i>	—	10,700	140	—	60	—	—	—
„ <i>quadricauda</i>	—	—	1,400	—	60	—	—	—
<i>Ankistrodesmus falcatus</i>	1,320	—	70	—	180	—	90	—
<i>Ulothrix zonata</i>	—	—	—	—	—	—	—	800
„ <i>subtilissima</i>	—	—	—	—	—	—	—	1,000
<i>Cladophora glomerata</i>	—	100	—	—	—	300	—	1,000
<i>Oedogonium</i> sp.	—	—	—	—	—	—	—	—
<i>Vaucheria sessilis</i>	—	—	—	—	—	—	—	—
<i>Oscillatoria brevis</i>	—	—	—	—	20	—	—	—
<i>Phormidium foveolarum</i>	—	+	—	—	—	+	—	—
<i>Sporotetras pyriforme</i>	—	21,900	—	—	—	1,100	—	—
<i>Stigeoclonium farctum</i> var. <i>riculare</i>	—	10,000	—	40,900	—	—	—	21,500
<i>Stigeoclonium falklandicum</i>	—	—	—	—	—	—	—	16,100
<i>Gongosira incrustans</i>	—	—	—	3,000	—	+	—	2,400
<i>Uvella frequens</i>	—	14,700	—	8,600	—	8,200	—	28,400
<i>Sphaerobotrys fluvialis</i>	—	2,700	—	—	—	+	—	3,800
<i>Chamaesiphonopsis regularis</i>	—	700	—	—	—	+	—	2,500
<i>Chamaesiphon curvatus</i>	—	500	—	—	—	—	—	—
<i>Oncolyrsa cesatiana</i>	—	1,500	—	—	—	1,100	—	—
<i>Heterolagnion Oedogonium</i>	—	400	—	700	—	—	—	+

TABLE X (continued).

Comparison of Sessile and Free-floating Communities—River Tees at Eryholme.

Date of collecting—	18. 11. 29		24. 3. 30		23. 4. 30		22. 5. 30	
No./sq. cm. of sessile algae	Fr.-fl.	1,300	Fr.-fl.	17,000	Fr.-fl.	2,200 <sup>1</sup>	Fr.-fl.	8,950,000
No./100 c.c. of free-floating algae	1,540	Sess.	15,000	Sess.	18,240	Sess.	14,590	Sess.
<i>Cyclotella Meneghiniana</i> . . . . .	30	—	100	—	150	—	—	—
<i>Meridion circulare</i> . . . . .	—	—	200	250	240	120	20	40,000
<i>Diatoma vulgare</i> . . . . .	+	—	400	600	720	120	600	45,000
<i>elongatum</i> . . . . .	—	—	—	250	150	90	200	160,000
<i>Synedra Ulna</i> . . . . .	40	50	700	—	270	25	120	54,000
<i>Acus</i> . . . . .	—	—	—	—	360	10	20	—
<i>tenera</i> . . . . .	—	—	—	—	540	140	120	+
<i>Ceratoneis Arcus</i> . . . . .	—	60	—	—	300	40	—	—
<i>Achnanthes exilis</i> . . . . .	—	—	—	—	—	40	—	—
<i>linearis</i> . . . . .	—	580	—	—	270	40	—	50,000
<i>microcephala</i> . . . . .	—	20	—	—	270	40	60	—
<i>Cocconeis placentula</i> . . . . .	270	190	20	—	1,200	80	180	86,000
<i>Navicula viridula</i> . . . . .	560	160	1,900	5,600	6,270	480	6,100	2,100,000
<i>cryptocephala</i> . . . . .	—	—	—	—	330	10	140	205,000
<i>rhyncocephala</i> . . . . .	30	—	—	550	930	—	—	—
<i>Gomphenema constrictum</i> . . . . .	—	—	100	—	240	25	—	14,000
<i>olivaceum</i> . . . . .	—	—	1,000	1,400	1,960	240	1,060	240,000
<i>Cymbella delicatula</i> . . . . .	+	—	—	—	330	+	240	10,000
<i>ventricosa</i> . . . . .	80	40	—	500	1,920	470	3,960	1,200,000
<i>Nitzschia palea</i> . . . . .	50	130	600	1,000	1,770	30	600	145,000
<i>acicularis</i> . . . . .	—	+	—	500	300	+	80	186,000
<i>dissipata</i> . . . . .	—	—	100	500	1,080	25	120	73,000
<i>sigma</i> . . . . .	—	—	300	—	—	—	—	+
<i>Surirella ovata</i> . . . . .	—	—	400	3,200	300	20	300	4,000,000
<i>Scenedesmus obliquus</i> . . . . .	—	—	—	—	—	—	—	10,000
<i>quadricauda</i> . . . . .	—	—	—	—	—	—	20	—
<i>Ankistrodesmus falcatus</i> . . . . .	—	—	—	—	—	—	—	—
<i>Ulothrix zonata</i> . . . . .	—	—	100	100	—	—	—	27,000
<i>subtilissima</i> . . . . .	—	—	—	—	180	—	—	—
<i>Cladophora glomerata</i> . . . . .	—	—	—	—	90	120	—	—
<i>Oedogonium</i> sp. . . . .	—	—	—	—	—	—	—	—
<i>Vaucheria sessilis</i> . . . . .	—	—	—	—	—	—	—	—
<i>Oscillatoria brevis</i> . . . . .	50	—	200	—	90	—	—	—
<i>Phormidium foveolarum</i> . . . . .	—	—	—	—	—	—	—	—
<i>Sporotetras pyriforme</i> . . . . .	—	—	—	—	—	—	—	—
<i>Stigeoclonium farctum</i> var. <i>reticulare</i> . . . . .	—	—	—	—	—	—	—	—
<i>Stigeoclonium falklandicum</i> . . . . .	—	—	—	—	—	—	—	—
<i>Gongosira incrustans</i> . . . . .	—	—	—	—	—	—	—	—
<i>Ulvella frequens</i> . . . . .	—	—	—	—	—	—	—	—
<i>Sphaerobotrys fluviatilis</i> . . . . .	—	—	—	—	—	—	—	—
<i>Chamaesiphonopsis regularis</i> . . . . .	—	—	—	—	—	—	—	—
<i>Chamaesiphon curvatus</i> . . . . .	—	—	—	—	—	—	—	—
<i>Oncobyrsa cesatiana</i> . . . . .	—	—	—	—	—	—	—	—
<i>Heterolagynium Oedogonium</i> . . . . .	—	—	—	—	—	—	—	—

<sup>1</sup> This frame was overturned *in situ*. A slide fourteen days after this date gave 96,500 organisms/sq. cm.

TABLE XI.

*Sessile and Free-floating Communities compared—River Tees at Yarm.*

Date of collecting—	15. 7. 29		19. 8. 29		16. 9. 29	
No. of free-floating algae/100 c.c. ,, ,, sessile algae/sq. cm.	100,700 Fr.-fl.	Sess. 523,000	5,490 Fr.-fl.	Sess. 366,000	9,650 Fr.-fl.	Sess. 9,240
<i>Cyclotella Meneghiniana</i> . . . . .	20,000	8,800	2,820	+	7,810	410
<i>Fragilaria intermedia</i> . . . . .	—	74,100	—	—	—	—
<i>Meridion circulare</i> . . . . .	—	—	—	—	—	—
<i>Diatoma vulgare</i> . . . . .	1,000	8,800	—	—	30	285
„ <i>elongatum</i> . . . . .	—	—	—	—	—	—
<i>Synedra Ulna</i> . . . . .	—	1,560	—	—	+	60
„ <i>Aeus</i> . . . . .	1,200	—	—	—	—	—
„ <i>tenera</i> . . . . .	3,200	—	—	3,500	—	—
<i>Ceratoneis Arcus</i> . . . . .	—	+	—	—	—	—
<i>Achnanthes exilis</i> . . . . .	—	—	—	1,500	170	—
„ <i>linearis</i> . . . . .	—	5,500	—	—	—	80
„ <i>microcephala</i> . . . . .	—	1,000	—	—	—	—
<i>Cocconeis placentula</i> . . . . .	1,200	247,500	480	126,000	660	7,150
<i>Navicula viridula</i> . . . . .	—	16,000	180	—	100	140
„ <i>atomus</i> . . . . .	800	—	—	—	—	—
„ <i>cryptocephala</i> . . . . .	400	40,000	—	—	—	—
„ <i>gracilis</i> . . . . .	—	+	160	—	20	—
„ <i>anglica</i> . . . . .	—	10,000	—	—	—	—
<i>Gomphenema constrictum</i> . . . . .	—	+	+	—	+	—
„ <i>olivaceum</i> . . . . .	—	—	140	—	70	30
<i>Cymbella ventricosa</i> . . . . .	7,200	29,000	140	5,500	310	100
„ <i>delicatula</i> . . . . .	—	—	40	—	90	—
<i>Nitzschia palea</i> . . . . .	2,540	5,000	300	23,000	200	100
„ <i>acicularis</i> . . . . .	3,310	4,000	140	—	70	—
„ <i>dissipata</i> . . . . .	—	—	80	—	50	—
„ <i>sigma</i> . . . . .	2,000	6,000	230	—	50	60
<i>Surirella ovata</i> . . . . .	—	+	20	—	—	—
<i>Scenedesmus obliquus</i> . . . . .	19,800	108,000	260	—	—	—
„ <i>quadricauda</i> . . . . .	20,000	6,000	440	—	—	30
<i>Ankistrodesmus falcatus</i> . . . . .	4,000	3,000	—	—	—	—
<i>Cosmarium Turpinii</i> . . . . .	2,600	—	40	—	—	—
<i>Ulothrix zonata</i> . . . . .	800	—	—	—	—	—
„ <i>subtilissima</i> . . . . .	—	—	—	—	—	—
<i>Uthella frequens</i> . . . . .	—	2,000	—	94,000	—	60

Dominant organisms are shown in heavy figures. + = only one individual seen in count.

TABLE XI (continued).

*Sessile and Free-floating communities compared—River Tees at Yarm.*

Date of collecting—	24. 3. 30		23. 4. 30		22. 5. 30	
No. of free-floating algae/100 c.c.	3,440	Sess.	13,070	Sess.	37,480	Sess.
„ „ sessile algae/sq. cm.	Fr.-fl.	8,870	Fr.-fl.	18,400	Fr.-fl.	1,177,000
<i>Cyclotella Meneghiniana</i> . . . . .	40	—	40	—	560	—
<i>Fragilaria intermedia</i> . . . . .	—	—	—	—	—	+
<i>Meridion circulare</i> . . . . .	100	160	540	200	120	+
<i>Diatoma vulgare</i> . . . . .	60	180	720	550	1,440	10,000
„ <i>elongatum</i> . . . . .	40	240	60	550	1,080	12,000
<i>Synedra Ulna</i> . . . . .	220	+	240	250	360	—
„ <i>Acus</i> . . . . .	—	40	—	—	2,200	6,000
„ <i>tenera</i> . . . . .	20	—	640	—	—	+
<i>Ceratoneis Arcus</i> . . . . .	100	—	300	100	160	6,000
<i>Achnanthes exilis</i> . . . . .	20	—	100	+	160	10,000
„ <i>linearis</i> . . . . .	100	—	—	400	160	28,000
„ <i>microcephala</i> . . . . .	—	—	40	—	—	—
<i>Cocconeis placentula</i> . . . . .	160	100	140	200	800	36,000
<i>Navicula viridula</i> . . . . .	900	2,390	4,440	6,640	7,180	232,000
„ <i>atomus</i> . . . . .	—	120	—	—	360	8,000
„ <i>cryptocephala</i> . . . . .	180	590	660	1,000	320	231,000
„ <i>gracilis</i> . . . . .	—	—	—	300	160	4,000
„ <i>anglica</i> . . . . .	—	—	—	1,000	—	—
<i>Gomphenema constrictum</i> . . . . .	—	—	+	—	—	—
„ <i>olivaceum</i> . . . . .	560	250	1,240	2,000	3,520	47,000
<i>Cymbella ventricosa</i> . . . . .	100	50	920	1,100	4,320	81,000
„ <i>delicatula</i> . . . . .	—	+	180	+	680	54,000
<i>Nitzschia palea</i> . . . . .	420	480	600	860	2,260	54,000
„ <i>acicularis</i> . . . . .	20	180	—	200	6,280	14,000
„ <i>dissipata</i> . . . . .	80	—	660	360	440	29,000
„ <i>sigma</i> . . . . .	20	100	180	400	80	4,000
<i>Surirella ovata</i> . . . . .	—	1,660	540	360	440	293,000
<i>Scenedesmus obliquus</i> . . . . .	—	—	—	—	—	—
„ <i>quadricauda</i> . . . . .	—	—	—	—	—	—
<i>Ankistrodesmus falcatus</i> . . . . .	—	—	—	—	—	—
<i>Cosmarium Turpinii</i> . . . . .	—	—	—	—	—	—
<i>Ulothrix zonata</i> . . . . .	60	130	140	+	120	+
„ <i>subtilissima</i> . . . . .	—	—	—	+	—	—
<i>Ulothrix frequens</i> . . . . .	—	—	—	—	—	—

Dominant organisms are shown in heavy figures. + = only one individual seen in count.

TABLE XII.

*River Lark. Species Present in Greater Numbers than 10/c.c.*

Date.	At Lackford.	Other places.
May 4, 1926	<i>Synedra Ulna</i> (38) <i>Euglena viridis</i> (15) <i>Sphaerotilus natans</i> (12)	<i>Synedra Ulna</i> (111) (333) <i>Nitzschia palea</i> (58) " <i>acicularis</i> (63) <i>Euglena viridis</i> (92)
June 6, 1926	<i>Synedra Ulna</i> (159) <i>Nitzschia palea</i> (47) " <i>acicularis</i> (34) <i>Sphaerotilus natans</i> (12)	<i>Synedra Ulna</i> (144) " <i>radians</i> (98) (100) <i>Nitzschia palea</i> (11) " <i>acicularis</i> (22) <i>Navicula viridula</i> (20)
July 20, 1926	None	<i>Synedra Ulna</i> (28) " <i>radians</i> (12) <i>Closterium Ehrenbergii</i> (20)
September 12, 1926	<i>Synedra Ulna</i> (198) <i>Diatoma vulgare</i> (14)	<i>Synedra Ulna</i> (14) <i>Nitzschia palea</i> (11)

(b) *The River Lark.*

The data derived from the River Lark (Butcher, Pentelow, and Woodley (l.c.)) confirms the conclusions from the Tees. Here, again, there is no counterpart of the *Ulvella-Cocconeis* community and no definite community of green algae; the only two recorded among the dominant algae were: *E. viridis* and, once only, *C. Ehrenbergii*. The dominant diatoms are *S. Ulna* and *N. palea*, which compare definitely with the dominant algae of the sessile community although the proportions differ. This difference is probably due to *S. Ulna* being more strongly attached than *N. palea*, and therefore it will be present in smaller numbers.

Sources for the potamoplankton in the Lark other than the river-bed may be:

1. An ornamental pond a mile above Lackford.
2. Ditches and drainage dykes along the river.
3. A sewage outfall fifty yards above Lackford.

*S. natans* and *E. viridis* are obviously introduced by the sewage effluent; *C. Ehrenbergii* is a rather ubiquitous desmid and was collected several times among *S. natans* in the sewage outfall. In any case it represents a very small proportion of the total potamoplankton and none of the organisms suggest recruitment from heleoplankton. This absence of unattached forms is all the more remarkable in view of the 'reed-choked' reaches of the river for seven miles above Lackford (see (11)).

Collections of similar composition are recorded by Fritsch (15) from the Cam, where the commonest species are *S. Ulna*, *N. lanceolata*, *N. exilis*,

and *M. varians*. In both the Tees and the Lark the non-attached Chlorophyceae, whatever their origin, play a very unimportant role. In some British rivers there are, however, in summer a considerable number of Chlorophyceae, viz. *Scenedesmus*, *Tetraspora*, *Ankistrodesmus*, *Pediastrum*, *Coelastrum*, and some desmids. The best examples in England are afforded by the Wharfe and the Thames.

(c) *The River Wharfe.*

The phytoplankton of this river has been investigated by Butcher (8) and Schroeder (38). The latter reports on collections made at Grassington, Pool, and Ulleskelf from June to September, 1926; my own results are based on monthly samples collected for a period of two years from Harewood, four miles below Pool.

My lists indicate (1) a *Diatoma-Nitzschia* community; (2) a *Melosira varians*-*Navicula viridula* community. Although there are no data available for the sessile communities, there are obvious similarities with the communities of the Tees. Schroeder's (38) lists indicate similar communities in the three stations of the Wharfe where he collected. *Melosira varians* is absent, but *C. pediculus*, *C. scutellum*, *A. parvula*, *R. curvata*, and *A. ovalis* all undoubtedly epiphytic, and *N. lanceolata*, which is probably a cosmopolitan form in this river as it is in the Lark.

The communities of green algae present in the summer are more marked in this river than in the Tees or Lark. The dominant species are *S. obliquus* and *S. quadricauda*, which seem to be among the commonest in all rivers and in all kinds of habitat, i.e. sessile, benthic, littoral, and free-floating. There are no others in Schroeder's lists, but others occasionally abundant at Harewood were *T. gelatinosa*, *P. Boryanum*, and *A. falcatus* and *G. monotaenium*. The nature of this community, and also the appearance of *Asterionella*, lead one to suspect a derivation from the limnoplankton of the three reservoirs in the Washburn valley which belong to the Leeds Corporation and have an outfall to the river below Pool and four miles from Harewood. Through the kindness of the City Waterworks Engineer several plankton collections were made from the reservoirs with a pump by myself and Mr. F. Barnet, and details of some of these are given in Table XIII comparing the river and one of the reservoirs.

It is possible that some of the species (e.g. *Gonatosygon*) in the Wharfe are derived from the reservoirs, but on the whole the two lists are very different and there is nothing to indicate that the communities of green algae are derived from this source, or that the algae so introduced persist for any length of time in the river as is definitely shown by Schroeder's lists.

TABLE XIII.

*River Wharfe. Dominant Organisms in the Plankton at Harewood and in Lindley Reservoir, Washburn Valley.*

<i>Month.</i>	<i>Harewood, 1921 and 1922.</i>	<i>Lindley Reservoir, 1922 and 1923. (Four miles above Harewood.)</i>
January	<i>Melosira varians</i> <i>Navicula viridula</i>	—
February	<i>Melosira varians</i> <i>Navicula viridula</i>	<i>Tabellaria fenestrata</i> <i>Ulothrix</i> sp.
March	<i>Diatoma vulgare</i> <i>Navicula viridula</i> <i>Synedra Ulna</i>	<i>Tabellaria fenestrata</i> <i>Ulothrix</i> sp.
April	<i>Diatoma vulgare</i> <i>Nitzschia palea</i> " <i>subtilis</i> <i>Amphora ovalis</i> <i>Synedra Ulna</i>	<i>Tabellaria fenestrata</i> <i>Asterionella gracillima</i> <i>Synedra affinis</i>
May	<i>Diatoma vulgare</i> <i>Nitzschia palea</i> " <i>subtilis</i> <i>Oscillatoria tenuis</i>	<i>Tabellaria flocculosa</i> <i>Synedra affinis</i> <i>Oscillatoria tenuis</i>
June	<i>Fragilaria virescens</i> <i>Scenedesmus quadricauda</i> " <i>obliquus</i> <i>Ankistrodesmus falcatus</i> <i>Gonatozygon monotaenium</i>	<i>Tabellaria fenestrata</i> <i>Asterionella gracillima</i> <i>Cyclotella comta</i>
July	<i>Tetraspora gelatinosa</i> <i>Gonatozygon monotaenium</i>	<i>Tabellaria fenestrata</i> <i>Asterionella gracillima</i> <i>Cyclotella comta</i>
August	<i>Scenedesmus obliquus</i> <i>Pediastrum Boryanum</i>	<i>Tabellaria fenestrata</i> <i>Asterionella gracillima</i> <i>Dinobryon acuminatum</i> <i>Ceratium hirundinella</i>
September	<i>Cocconeis Pediculus</i> <i>Rhoicosphenia curvata</i>	<i>Tabellaria fenestrata</i> <i>Asterionella gracillima</i> <i>Dinobryon acuminatum</i> <i>Gonatozygon monotaenium</i>
October	<i>Cocconeis Pediculus</i> <i>Closterium Ehrenbergii</i> <i>Melosira varians</i>	<i>Tetraspora gelatinosa</i> <i>Volvox globator</i> <i>Dinobryon acuminatum</i> <i>Gonatozygon monotaenium</i>
November	—	—
December	<i>Navicula viridula</i> <i>Melosira varians</i>	—



(d) *The River Thames.*

The portion of this river investigated by Fritsch (14) is rather different from those so far considered owing to the greater depth and the somewhat turbid character of the water, which makes the area where sessile algae can grow comparatively small. On the other hand, backwaters and pools are frequent, so that algae characteristic of such semi-stagnant waters may be expected to predominate. The scarcity of *Cocconeis* may be due either to the absence of sessile growths or to the current being too weak to detach it. The plankton of several backwaters was examined as a possible source of the plankton of the main river, but the collections are so varied that they afford no definite evidence of the relative value of the backwaters as a source of supply.

As there are no numerical data it is not easy to discover the relative value of the various communities. The diatoms, which are the most abundant of the algae, show definite similarities to the other rivers considered, and there seems to be a *Synedra Ulna-Melosira varians* community as in the Wharfe, and a *Nitzschia-Pleurosigma-Surirella* community.

It can be seen from the Tees and Lark results that *S. Ulna* is frequently epiphytic and *S. ovata* abundant on the river-bed and probably cosmopolitan. *N. sigmoides*, *P. attenuatum*, and *M. varians* may be derived either from the algal growths among the macrophytic vegetation along the banks—since diatoms are very frequent in such localities—or from the backwaters, or from some quiet muddy bottom as in other streams (see Table V).

The community of green algae again corresponds to what is found in the Wharfe, namely, a community with *Scenedesmus* spp. and *P. Boryanum* dominant. There is also in addition *Eudorina* and *Pandorina*. The *Cyclotella* community in the Tees seems to have its counterpart here in *Stephanodiscus Hantzschianus*.

On the whole the plankton of the Thames suggests derivation partly from the river-bed (*S. Ulna*), partly from the littoral zone (*P. attenuatum*), and partly from backwaters and pools, but there is too little evidence to draw definite conclusions.

II. *Rivers of the Continent.*

The continental rivers may be divided into (1) those which are sufficiently shallow to allow light to penetrate on to the bottom and correspond to the majority of those found in this country, and (2) large and deep rivers with very little light reaching the river-bed and in which therefore the bottom flora is sparse.

Among those of the first group the most completely investigated case is that of the Limmat in Switzerland by Limanowska (l.c.). Her lists show that the Limmat contains a great abundance of the same algae as are

found in the English rivers, viz. great abundance and diversity of species of the genera *Gomphonema*, *Navicula*, *Cymbella*, and *Stigeoclonium*. Abundance of *D. vulgare*, *Cl. pediculus*, and *C. glomerata*. She also records the diatoms characteristic of potamoplankton, viz. *Tabellaria fenestrata* and *Asterionella gracillima* as being richly represented. Limanowska also points out that many of these are abundant as epiphytes or on stones, shore, and macrophytes, and her brief description of the periodicity of these attached and bottom-living forms accords well with what has so far been established in English streams.

The Havel investigations by Bethge (4) and Krieger (l.c.) are confined to potamoplankton. The Havel possesses quite an individual character owing to the numerous lake-like expansions in the course of the river, and Krieger's statement that the potamoplankton is determined (in the narrower sense) by the limnoplankton developed in the lakes, though true for the Havel, is by no means of universal application, since the majority of rivers do not show the peculiar features of this German stream.

Of the large, deep rivers of the second group a great many have been investigated both on the continent and in other parts of the world. For consideration in this paper only some of the most comprehensive investigations are selected :

Danube	Brunnthaler (7)
Elbe	Schorler (36)
Oder (near Breslau)	Schröder (37)
Rhine	{ Lauterborn (24)
	{ Kolkwitz (22)
Weser	Lemmermann (25)
Volga	Bolochonzew (6).

Many of the accounts lack certain data that are necessary for a full elucidation of the microflora of a river. In the first place very few of these provide quantitative data, so that no estimate can be made of the relative importance of the various organisms. Again, many authors exclude from their lists certain of the free-floating forms of epiphytic or benthic origin presumably present in most collections. Lemmermann (26), for instance, in summarizing the lists of other workers from the point of view of river plankton, only considers what he regards as true plankton forms and excludes all organisms known to be epiphytic, although in another paper (25) he gives a long list of such organisms that he collected in the Weser. No doubt others have adopted the same procedure of omitting obvious 'epiphytes'.

From the point of view developed in this paper, however, it is the relative quantities of epiphytic, benthic, and planktonic forms in the free-floating community which are important. The plankton-lists from all the

above-mentioned rivers include cosmopolitan forms such as *S. Ulna*, *D. vulgare*, *Nitzschia* and *Navicula* spp. which, as my results show, undoubtedly multiply to a very great extent on the river-bed. That being the case it does not seem impossible that such closely allied genera as *Fragilaria*, *Tabellaria*, and even *Asterionella* also reproduce to a considerable extent in the same habitat.

Few of the papers above cited make any reference to the sessile community, so that it is uncertain whether it exists at all or how far it is developed. In one of the papers dealing with the Volga, however (Bolochoziew (6)), the phytoplankton is classified under three headings, viz.:

1. True plankton organisms which pass all their life suspended in water, e.g. *Volvox*, *Pediastrum*, *Asterionella*.

2. Bottom plankton organisms which are found as frequently on the river-bed as suspended in the water, e.g. *S. obliquus*, *C. comta*.

3. Chance plankton organisms which are normally to be found in the littoral zone but are carried into the plankton during floods, e.g. *A. ovalis*. These respectively correspond in part to my groups (*f*), (*e*), and (*d*) (cf. p. 833). Bolochoziew's conclusion confirms the view that very little of the potamoplankton is euplanktonic, since most of the species listed by other authors, particularly when working on small streams, are those placed by him among the 'bottom plankton' forms.

From the rest one can glean from the papers certain facts which are more or less true of rivers generally. There is always an abundant diatom flora which in every case includes *S. Ulna* (or some variety of it), *D. vulgare*, *Fragilaria* spp., *N. acicularis*, and *Cyclotella* spp. Where littoral or epiphytic diatoms are included in the lists, certain genera, viz. *Cocconeis*, *Navicula*, and *Amphora* are very common. Secondly, the lists of Chlorophyceae and Myxophyceae collected in the summer plankton almost always include as the commonest members species of *Scenedesmus*, *Ankistrodesmus*, *Pediastrum*; and *Microcystis aeruginosa*. These are important members of the group of organisms which appear so spasmodically in the Tees and which are included in Bolochoziew's group of bottom plankton forms.

A third important point is the scarcity of undoubted euplanktonic forms such as *Ceratium*, *Synura Uvella*, and Desmidiaceae similar to those that are characteristic of pools or lakes. Such forms are recorded, but usually where a river is linked up with waters of the latter type.

It seems possible to draw the following general conclusions in respect to the potamoplankton and its original.

- (1) The potamoplankton can be divided into two groups: (*a*) The diatoms of which the commonest recorded are *Fragilaria* and *Synedra*; (*b*) the small green and blue-green algae, of which *Scenedesmus* and *Pediastrum* are the most frequent and widely distributed.

(2) The second of these two groups becomes the more important as the river becomes larger.

(3) The commonest organisms of the potamoplankton are ubiquitous and can be found developing in a variety of habitat, namely, pond, lake, marshy places, or river-bed.

(4) In the case of large rivers the relation between the potamoplankton and its sources of supply (namely, lakes, pools, bogs, backwaters, and river-bed) cannot be established from the data so far published.

(5) In smaller streams there is a certain amount of evidence that, as in those studied by me, the sessile flora constitutes the most important source of supply and largely determines the nature of the phytoplankton.

#### GENERAL CONCLUSIONS.

It may be concluded from the above considerations that in streams and small rivers where there is sufficient light penetrating to the river-bed, a great many organisms in the potamoplankton are derived from the algal communities of the bottom.

Of the categories mentioned on p. 833 plants of the stalked diatom type, i.e. those possessed of only a basal attachment, and of the unattached type, i.e. those without definite means of attachment, are most commonly found in the plankton, since they are most easily washed away, e.g. *S. Ulna*, *C. ventricosa*, *Diatoma* spp., and *Navicula* spp. On the other hand, those of the thalloid type, although abundant on the river-bed, are so firmly attached along a large part of their surface (e.g. *Ulvella*, *Stigeoclonium*, *Oncobyrsa*) that they are rarely washed off as complete plants. Forms such as *Chamaesiphon* which have a basal attachment also are not readily detached because of the smallness and nature of the aggregates. In larger rivers more members of the unattached, motile, and stalked-diatom types appear in the plankton. These are the forms without obvious attachment, but some of them are often found abundantly on the river-bed and among the macrophytes (e.g. *Cyclotella*, *Pediastrum*, and *Scenedesmus obliquus*). In the very large continental rivers these constitute a characteristic group.

Inasmuch as all river plankton must be continuously recruited it is important to determine the source of the constituent organisms, as only by studying the conditions at such places in relation to the general changes in the river, can a true understanding of seasonal variation be obtained.

With very few exceptions all the organisms in the potamoplankton can be found at one time or another on the river-bed and among the littoral and submerged macrophytes. The question that arises is whether forms met with on the river-bed have become caught and lodge there as they drift downstream (i.e. they are adventive), or whether they normally grow

and multiply in this habitat and individuals pass from it to supply the constituents of the potamoplankton. That a certain proportion of all the organisms involved are caught up is supported by the absence of special attaching organs in a considerable number of them, and by the fact that such forms become more abundant in the larger and deeper rivers. Though there is little evidence in the literature that in other types of water they normally occur attached, such forms may be found in tremendous numbers on the surface of the mud in ponds or in shallow streams where the current is small, or attached to the macrophytic vegetation where again they are protected from the rigours of the greatest currents and the loss is less than the gain by multiplication.

No conclusive evidence can be based on the multiplication of individuals of this type which can be seen to take place either in the plankton or in the sessile community. What takes place while the organisms are drifting downstream is of value only to the lower reaches, and it is to be anticipated that where division takes place extensively there will be a marked increase in planktonic forms. Multiplication on the river-bed only indicates that here the organisms find conditions not too unfavourable, but if this multiplication is shown to be extensive then it is an indication that the organisms concerned find a favourable habitat in which they can contribute to the numbers of the plankton, no matter where their original source.

It is difficult to make a numerical comparison between river-bed and free-water, but in Tables IX–XI have been given the numbers of attached organisms per square centimetre found on slides in the River Tees and the plankton organisms in 100 c.c. of water at the same time and place. The basis on which this comparison rests is stated on p. 850. The figures show generally an obvious relation in the dominant plants of the two groups, although there are very great differences at times in their numerical representation. In the majority of cases the organisms from the river-bed seem to be there in greater numbers than those in the water, though at times the reverse is the case. The greatest differences are, however, to be observed at the times of greatest change of the microflora, namely, in March, June, and September, and in the main among the forms without visible means of attachment, and this is the group which presents the greatest difficulties in elucidating their origin. *S. obliquus*, as an example, occurs in quantity both on the river-bed and in the plankton; sometimes earlier and sometimes later in the former than in the latter, and it does not show the same close numerical relationships as are seen in the case of *Cocconeis* and *N. viridula* for instance.

There is the possibility that these cosmopolitan forms such as *Scenedesmus* are equally well able to reproduce in ponds and ditches and on the river-bed, making it impossible by an occasional collection to come to a fair

conclusion as to the source of these constituents of the potamoplankton. Even if the forms in question are only such as have been caught up on the river-bed they are for the time being arrested in their journey downstream, and thus retained as a future source of potamoplankton. It will be necessary to assemble far more data as to the potamoplankton, the algal growth among the macrophytes, and that on the river-bed before this problem can be solved.

An intimate relation between the sessile and free-floating algae of a river undoubtedly exists. The shallower the river the larger the area of river-bed exposed to sufficient light to support a growth of green plants and the larger therefore the number of sessile algae able to act as a source of supply for the plankton.

Since a great many of the algae in the potamoplankton are epiphytes derived from the river-bed and other external sources, the designation 'plankton' seems rather misleading. The tendency among those dealing with river plankton has been to disregard obvious epiphytes and other adventitious forms and to exclude them in considering the general problem of plankton. Yet among the forms retained there is a large number of algae whose optimum mode of life is undoubtedly an attached one, e.g. *S. Ulna*, *D. vulgare*, &c., or one on the shore or surface of the mud (i.e. a benthic one), e.g. *N. viridula*. Further, it seems impossible to draw a distinct line of demarcation between obvious epiphytes or attached forms, obvious planktonts, and the large number of cosmopolitan forms which may belong to either category.

It would therefore seem best to put into one group all the organisms and to distinguish among the algae of a river two sub-groups, namely, those that are collected floating freely in the water and those that are collected from the growths or deposits on the river-bed or on any object in the water including the macrophytes.

Zacharias's conception of the potamoplankton should be enlarged to include all the organisms of the first group as has been done in this paper, since it seems obvious that there are no truly pelagic forms in flowing water. In the work on the River Lark (l.c.) the term 'free-floating microflora' was used to avoid, if possible, ambiguity, as several workers have attempted to narrow down the above conception of plankton.

For the second group the term 'sessile microflora' has been used in a previous paper (l.c. (11)), indicating that the organisms remain attached or 'seated' on other objects. This group may be further sub-divided into (a) organisms with obvious means of attachment including epiphytes, and which might be termed the 'anchored forms'; (b) bottom-living forms without visible attachments; and (c) cosmopolitan forms met with in a great many different habitats and common to both of the larger groups. All these groups may include a variety of biological types and growth forms

as outlined on p. 833 and considerable more data are required before one can classify these organisms more definitely.

The data recorded from the River Tees were obtained in the course of a comprehensive survey of that river undertaken by the Ministry of Agriculture and Fisheries for the Department of Scientific and Industrial Research as part of the programme of the Water Pollution Research Board. The author wishes to express his thanks to the Department for permission to publish the data utilized in this paper in advance of the detailed report which is in preparation. The author also desires to express his gratitude to Professor F. E. Fritsch and to Dr. W. H. Pearsall for many valuable suggestions.

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## EXPLANATION OF PLATES XXXIII AND XXXIV.

Illustrating Dr. Butcher's paper on Studies in the Ecology of Rivers. II.

Microphotographs of Algal Growths on Glass Slides.

Figs. 1, 2, 3, 4, 9, 10, 17, 18 × 200.      Figs. 5, 6, 7, 8, 11, 12, 13, 14, 15, 16 × 500.

Fig. 1. Diatom community in R. Lark at Lackford in March, 1928. *Synedra Ulna* (a), *Meridion circulare* (b).

Fig. 2. Diatom community in R. Cam at Shelford in March, 1928. *Synedra Ulna* (a), *Distema vulgare* (c).



Figs. 3 and 4. Mixed diatom-Cocconeis community in R. Cam at Jesus Lock in September, 1928. *Synedra Ulna* (a), *Diatoma vulgare* (c), *Cocconeis placentula* (d).

Fig. 5. Diatom community in R. Itchen at Alresford in March, 1926. *Nitzschia palea* (e), *Navicula gracilis* (f).

Fig. 6. Diatom community in R. Tees at High Force in April, 1930. *Achnanthes microcephala* (g), *Diatoma elongatum* (h), *Gomphonema olivaceum* (i), *Cymbella delicatula* (j).

Fig. 7. Diatom community in R. Balder at Cotherstone in April, 1930. *Cymbella ventricosa* (k).

Fig. 8. Diatom community in R. Tees at Eryholme in March, 1931. *Navicula radiosa* (l).

Fig. 9. Ulothrix community in R. Tees at High Force in May, 1931. *Ulothrix subtilissima* (m).

Fig. 10. Ulothrix community in R. Tees at Eryholme in May, 1931. *Ulothrix zonata*.

Fig. 11. Cocconeis-Ulvella community in R. Lark at Lackford in July, 1928. *Cocconeis placentula* (d), *Sphaerobotrys fluviatilis* (o), *Ulvella frequens* (p).

Fig. 12. Cocconeis-Ulvella community in R. Itchen at Alresford in July, 1929. *Cocconeis placentula* (d), *Sphaerobotrys fluviatilis* (o), *Ulvella frequens* (p).

Fig. 13. Cocconeis-Ulvella community in R. Tees at Rokeby in August, 1929. *Cocconeis placentula* (d), *Ulvella frequens* (p).

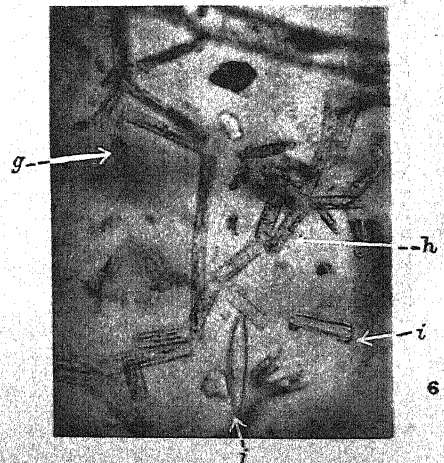
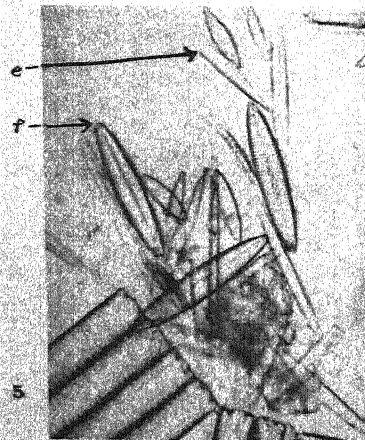
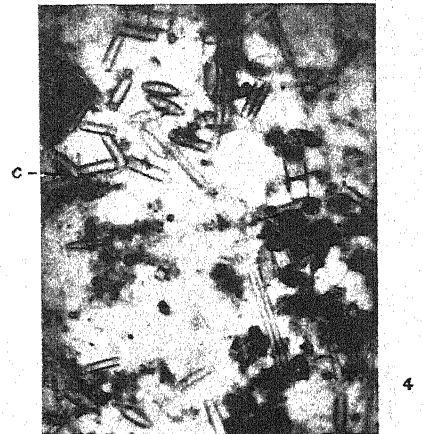
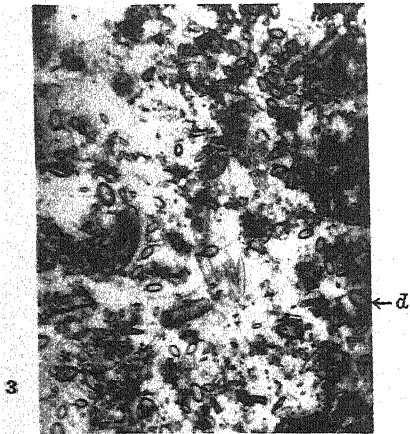
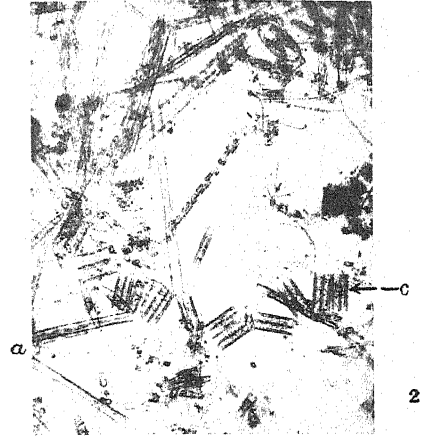
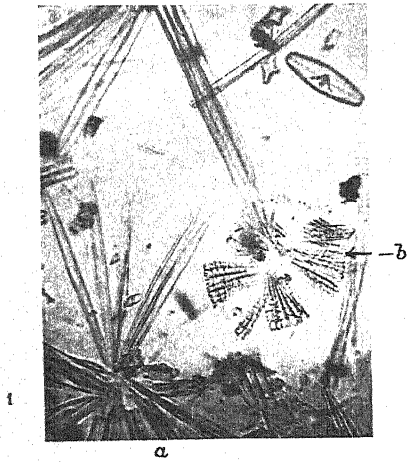
Fig. 14. Cocconeis-Ulvella community in R. Tees at Eryholme in August, 1929. *Cocconeis placentula* (d), *Scenedesmus obliquus* (q).

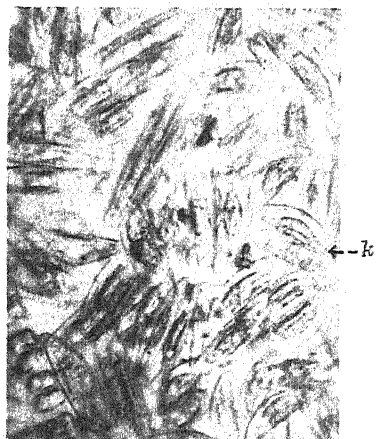
Fig. 15. Cocconeis-Ulvella community in R. Tees at Cotherstone in July, 1930. *Cocconeis placentula* (d), *Chamaesiphonopsis regularis* (r).

Fig. 16. Cocconeis-Ulvella community in R. Skerne north of Darlington in August, 1930. *Gongrosira incrustans* (s), *Chamaesiphon curvatus* (t).

Fig. 17. Cocconeis-Ulvella community in R. Tees at Croft in August, 1929. *Ulvella frequens* (p), *Chamaesiphon curvatus* (t), *Stigeoclonium farctum* (u).

Fig. 18. Cocconeis-Ulvella community in R. Tees at Eryholme in August, 1929. *Cocconeis placentula* (d), *Ulvella frequens* (p), *Stigeoclonium farctum* (u).

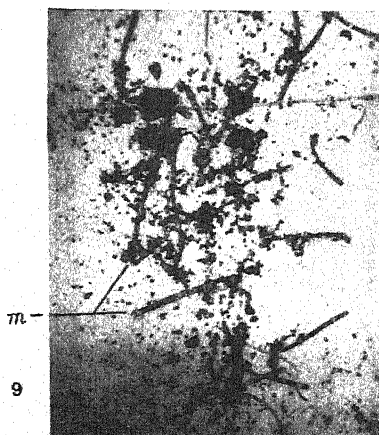




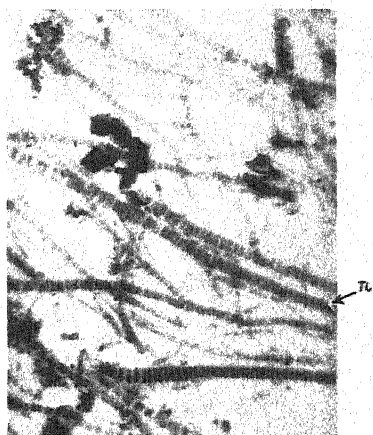
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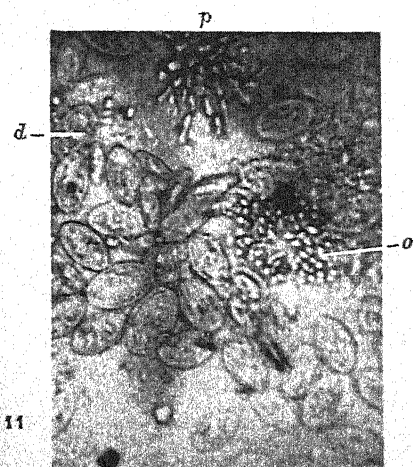
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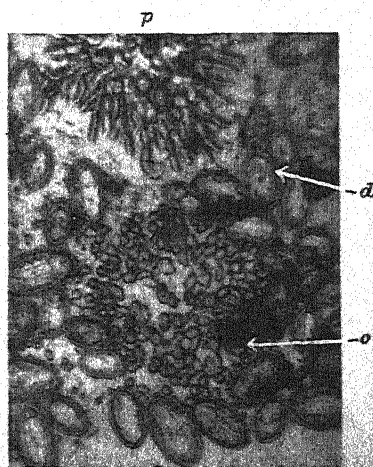
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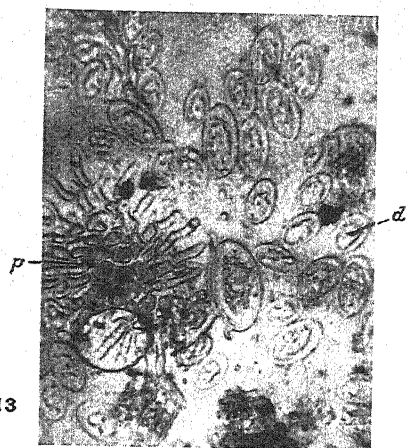
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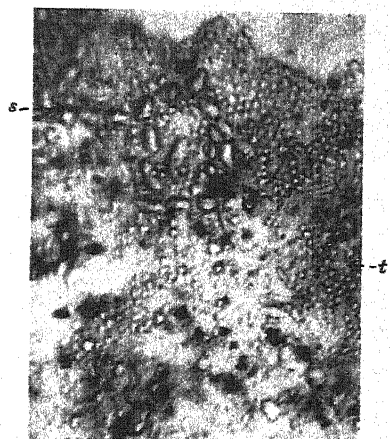
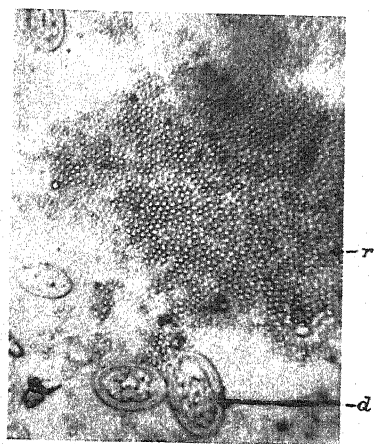
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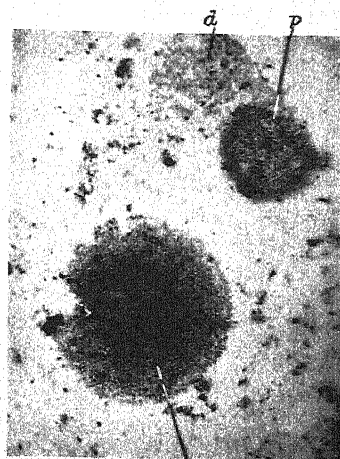
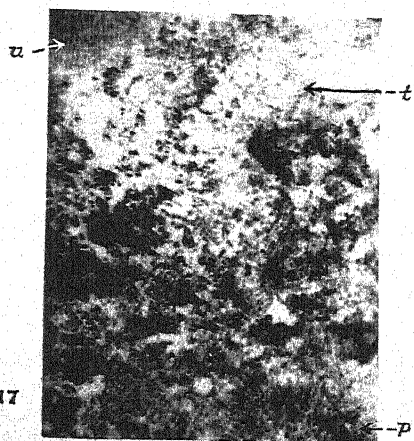
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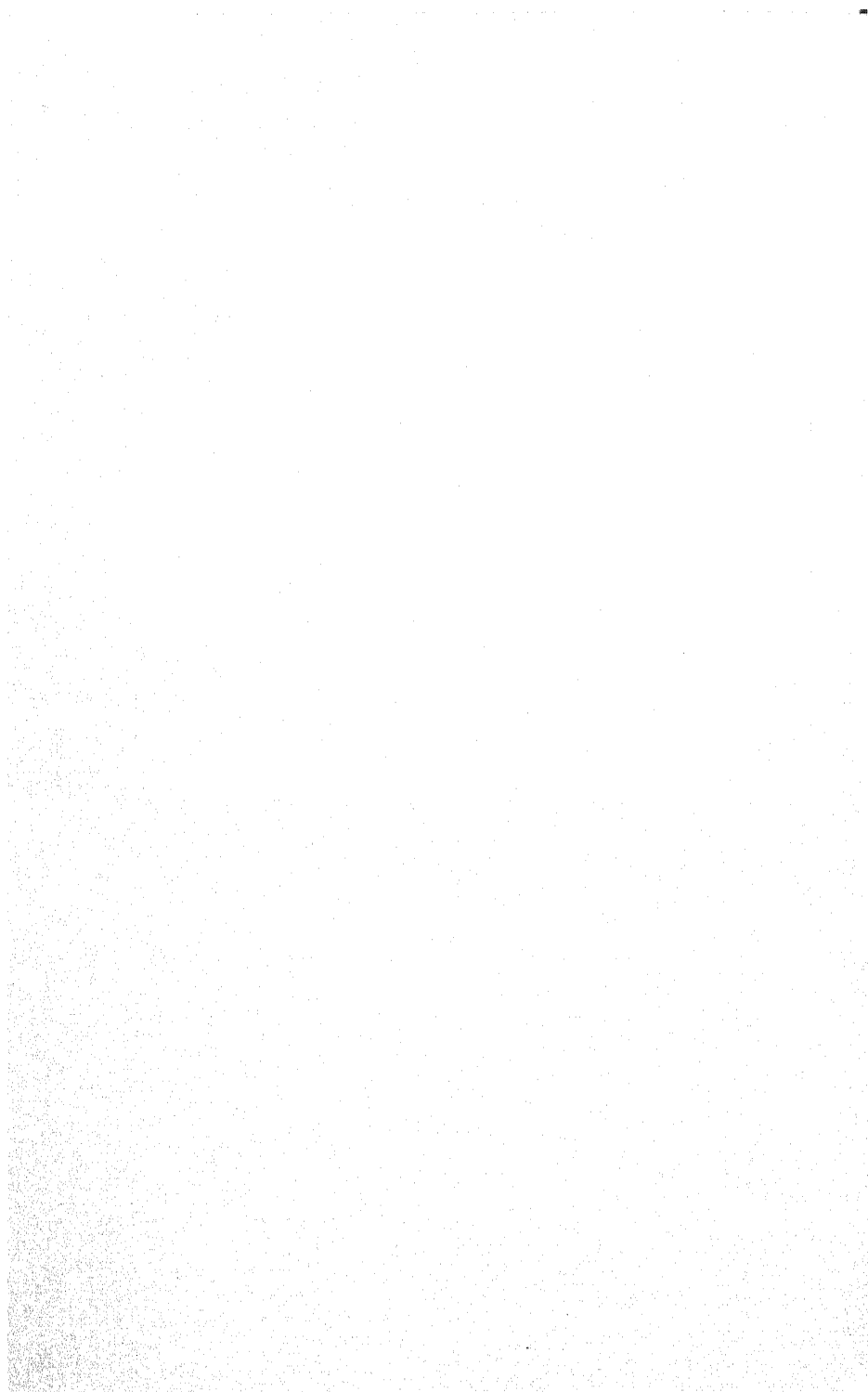
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# On a Palaeozoic Tree-fern, *Grammatopteris Baldaufi* (Beck) Hirmer, a Link between the Zygopterideae and Osmundaceae.

BY

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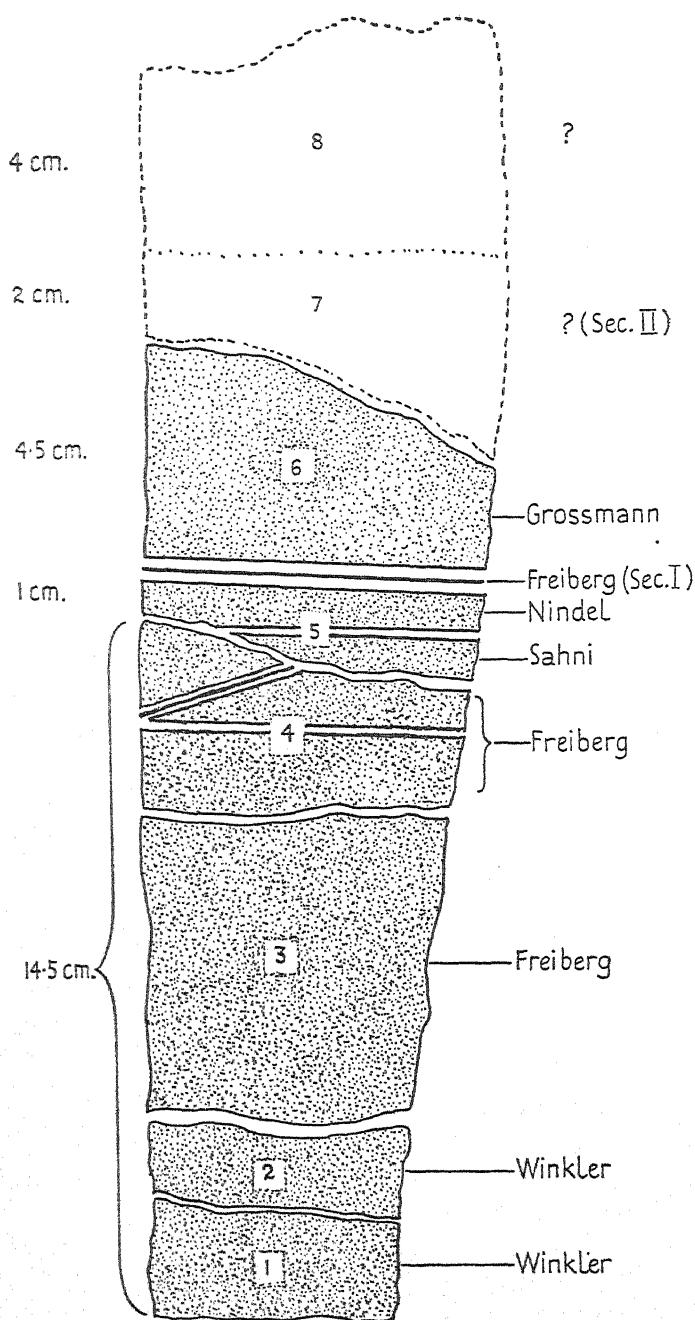
With Plate XXXV and five Figures in the Text.

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## INTRODUCTION.

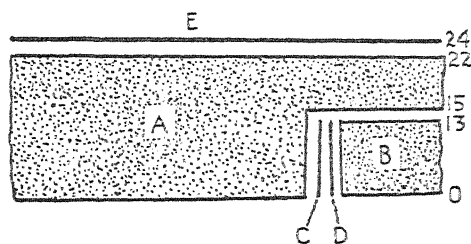
ONE of the main results of Kidston and Gwynne-Vaughan's classical researches on the Fossil Osmundaceae (3, pt. 3, pp. 663-4, pt. 4, pp. 466-73) was the discovery of facts pointing to a common origin of the Osmundaceae and Zygopterideae. It is unfortunate that during the lifetime of these authors the structure of *Grammatopteris* was only very imperfectly known; for although with their masterly treatment of the evidence they had already placed their theory on a sound footing, they did not live to witness the striking vindication of it which *Grammatopteris* provides. In a paper published in 1918 it was tentatively suggested (11,



TEXT-FIG. 1. The type-specimen. The numbers 1-8 indicate the fragments into which the stem was already broken when found; the lengths given are approximate.



p. 374) that in rocks older than those which have yielded the most primitive known osmundaceous remains (*Zaleskya* and *Thamnopteris*)<sup>1</sup> forms may yet be discovered which it would be difficult to assign to one or the other of



TEXT-FIG. 2. The British Museum specimen (nat. size), now in two blocks (A, B) and three thin sections (C, D, E). (Geol. Dept. Brit. Mus. V. 19,667). The numbers 15, 22, 24 indicate in millimetres the heights above the base (zero) of the specimen.

these families. Indeed, it may be that we already have one such form in *Grammatopteris Rigolloti*.<sup>2</sup> Our knowledge of the type-species has not advanced since Renault's time (10, p. 45) but a reinvestigation of an allied species recently discovered in Germany (1) has shown that the remarks just quoted were fully justified.

The material for the present investigation was obtained during two recent tours in Europe (1929-30), undertaken chiefly with the object of putting together the scattered fragments of certain silicified plants in the hope of examining them as far as possible in their entirety.

The type-specimen of *G. Baldaufi* was originally found in eight pieces (now scattered); the whereabouts of the different fragments, as far as I have been able to trace them, are indicated in Text-fig. 1. The fossil was discovered in July 1915 by a workman, the late Bruno Winkler, during the building of the Frankenberger Strasse at Hilbersdorf, a suburb of Chemnitz which has long been famous for its buried treasure of silicified plants. The two lowermost pieces (Nos. 1, 2) were inherited by Bruno's son Georg Winkler of Lübeck, and are now in his private collection. My attempt to borrow one of them for sectioning was unsuccessful, but in August 1930 Mr. W. N. Edwards showed me in the Geological Department of the British Museum the lower part of another stem of the same species which, through the kindness of the authorities and particularly of Mr. Edwards, I was able to examine in thin sections. At Freiberg, Prof. F. Schumacher, Director of the Bergakademie, very kindly lent me one of the two pieces of Winkler's specimen preserved there (No. 4), as well as the two transverse sections originally figured by Beck.<sup>1</sup> In Chemnitz, Herr F. Nindel, a druggist by profession well known for his interest in fossils, to whom we owe the first description of this important specimen (7, p. 71), generously

<sup>1</sup> The remaining sections described by Beck could not be found (July 1, 1930).

gave me a slice from a fragment (No. 5) in his possession; while Herr Baurat J. Grossmann kindly allowed me to examine the adjoining piece (No. 6). I have not been able to trace the two uppermost fragments (Nos. 7, 8).

#### PREVIOUS WORK.

Our previous knowledge of the species is confined to the papers by Nindel (7, pp. 71–2) and Beck (1) already cited. Recently Hirmer (5, p. 538) has transferred the species to *Grammatopteris*, a step which is no doubt justified (8, p. 138; 9, p. 111). Nindel only describes the gross features; he regards the plant as a new species of *Tubicaulis*, but does not suggest a specific name. A brief history of the fossil is given, but there are no figures. Beck published several good photographs, but his description is incomplete and inaccurate. Thus he does not mention the ramentum, nor the relation between the root- and leaf-traces. The dark zone of crushed thin-walled tissues round the foliar bundle is described as a sclerenchyma, while the phloem is indicated near the periphery of the petiolar cortex. He mistakes for adventitious roots certain masses of sclerenchyma in the stem cortex, and describes secondary wood, a tissue of which there is no trace in the stele. The new genus *Protothamnopteris* was no doubt created in ignorance of Renault's work.

#### DESCRIPTION.

##### *Habit.*

From Beck's description of Winkler's specimen it was clear that the plant was a small-sized tree-fern, like some of the fossil Osmundaceae. The London specimen shows for the first time that the base of the stem was covered by a felt of adventitious roots, which also concealed the petioles of some of the earliest leaves. The eccentric position of the stem in the root-felt (Pl. XXXV, Fig. 7) may either be due to incomplete preservation or it may indicate that (as in most modern tree-ferns) the juvenile stem was not quite erect, in which case the root-felt would be better developed on the side next the ground.

##### *Stem.*

*Stele*.—The stele is a solid cylinder varying from 3.75 to 5 mm. in diameter. The wood is differentiated into a stellate core and an outer zone with its margin more or less deeply invaginated in places. In the London specimen (Pl. XXXV, Figs. 7–10) the form of the stele is roughly pentagonal, and at least four of the angles have a ray of the central xylem pointing towards each. At three of the corners, moreover, incipient leaf-traces are seen at different stages of development. The whole appearance

strongly suggests that the leaf-traces arose in relation to the rays of the central xylem; and this may also be expected by analogy with other ferns in which there is a stellate core of pure or mixed xylem. At first sight the phyllotaxis seems to conform to a  $2/5$  system, but a closer inspection shows that the actual fraction is nearer  $5/13$  (see Pl. XXXV, Fig. 9).

This is the condition near the base of the stem. Higher up, where the leaves are more crowded (Winkler's specimen, Figs. 1, 5, 6) the pentagonal form of the stele is lost, the rays of the central xylem are much less distinct but more numerous, and the phyllotaxis approaches that seen in *Thamnopteris* and other Osmundaceae.

A few words are needed concerning the incisions in the outer xylem (Pl. XXXV, Figs. 5, 10 *inv.*). Although in the available sections they appear to have no evident relation with the leaf-traces, it is difficult to regard them in any other light except that they are incomplete leaf-gaps corresponding to those recently described by Zalessky in *Chasmatopteris* (18) where also the inner xylem is left intact. The incisions are distinctly more numerous in the higher parts of the stele where the leaf-traces are more crowded (Pl. XXXV, Fig. 5), than lower down where they are fewer (Pl. XXXV, Fig. 10). The fact that an incision is not seen in immediate relation to a leaf-trace need not mean that no relation exists. As the stele is solid and the leaf-trace, not being C-shaped, has no decurrent 'pocket' of ground tissue, the incision which it subtends would not come clearly into evidence till some distance above the point of departure of the trace. Pl. XXXV, Fig. 1, shows several incisions which lie opposite to leaf-traces slightly removed from the stele.

The xylem consists only of tracheides, without any thin-walled parenchyma. The cells of the central xylem are of two kinds. Most of them are ordinary elongated tracheides,  $40-80\mu$  across; their walls are finely pitted or reticulate, with occasional transitions to a multiseriate scalariform type.<sup>1</sup> Mixed with these are a few broad and short tracheides which lie in vertical rows and reach a diameter of  $160\mu$  or more (Pl. XXXV, Fig. 5, Text-fig. 5); the walls of these parenchymatous tracheides<sup>2</sup> have loose reticulate or scalariform thickenings. The outer xylem consists of ordinary tracheides which become rather narrower towards the periphery. In one or two places (e.g. Pl. XXXV, Fig. 5, right) there is a faint suspicion that the cells are radially arranged, but Beck was no doubt mistaken in describing the outer xylem as a secondary tissue (8, p. 138).

The position of the protoxylem is not easy to determine. As the elongated elements all show the same type of sculpturing, the only available criterion is the size of the tracheides, which is not always a reliable

<sup>1</sup> Cf. *Botryopteris* (18, p. 340) and *Tubicaulis Berthieri* (2, Pl. III, Fig. 19).

<sup>2</sup> Which probably have a water-storing function, like the similar cells in *Thamnopteris*, *Megaloxyton* (14) and other fossil genera.

guide. The finest elements are those in the central xylem, where they lie in scattered ill-defined groups (Pl. XXXV, Fig. 5) ; and the analogy already pointed out with other ferns having a stellate central xylem suggests that these groups are the protoxylems. This is the view here provisionally adopted. But narrow tracheides also occur along the periphery of the stele, though here there is no arrangement in groups.

The phloem and associated tissues are too badly crushed to admit of a clear description ; their remains are preserved as a thin dark band which closely invests the xylem, both of the stele and of the foliar strands.

*Cortex.*—The cortex of the stem is in three zones. The innermost is a homogeneous tissue of small thin-walled cells. The middle cortex has a ground-work of the same tissue, but there are also a few scattered cells of large size, apparently secretory sacs. A prominent feature of this zone is the crowded nests of sclerenchyma, already mentioned above (Fig. 6, *scl. z.*). Some of the nests include one or more unusually large cells placed at or near the centre. Beck (loc. cit., pp. 518–19) regarded these sclerotic nests as the resistant central parts of adventitious roots of which the delicate outer cortex was supposed to have decayed. As already stated, longitudinal sections show that these groups of sclerotic cells are not strands (Text-fig. 3), but isolated masses scattered in the parenchyma.

### Leaf.

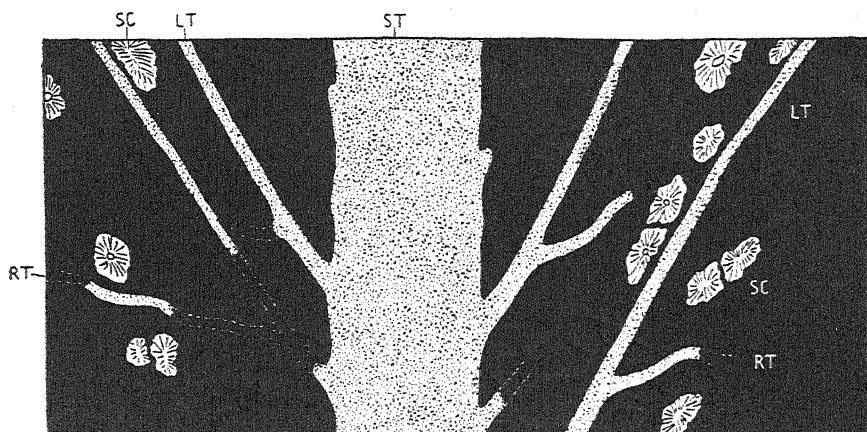
The leaf-bases are cylindrical, without the ‘stipules’ so characteristic of the Osmundaceae, both recent and fossil.

*Leaf-trace.*—At first the leaf-trace is inclined at about 30 degrees to the stele ; higher up it makes an angle of about 45 degrees. This seems to be a fairly regular occurrence (Text-fig. 3). In the inner cortex a root-strand usually comes off from the abaxial side of the leaf-trace.

Just above its origin the leaf-trace is elliptical in section, sometimes fusiform (Pl. XXXV, Fig. 15), with the major diameter well under a millimetre in length ; but it soon thins down to a tangential band. In the outermost leaves, which are cut at a height of 6–7 cm. above the point of emission of the trace, the band is about 2 mm. long.

The exact position of the protoxylem is not always easy to ascertain, owing partly to the fact that the pitting is not distinctive, partly to the inclination of the leaf-traces, which are very obliquely cut in a normal transverse section of the stem. The preservation also is rather imperfect. This at least is clear, that even in leaf-traces slightly removed from the stele, such as those shown in Pl. XXXV, Figs. 14, 15, the narrowest tracheides are not immersed as might be expected, but superficial. The archaic mesarch phase which is so characteristic of forms like *Thamnopteris*, *Asterochlaenopsis*, *Ankyropteris* and other primitive ferns is thus unrepresented. In Pl. XXXV, Fig. 15 the narrower elements lie in irregular positions all round the strand.

Higher up, where the bundle is a tangentially elongated band, the narrowest tracheides usually form two more or less clearly defined groups, one at each end of the band (Pl. XXXV, Fig. 16), as figured by Renault in *G. Rigol-*



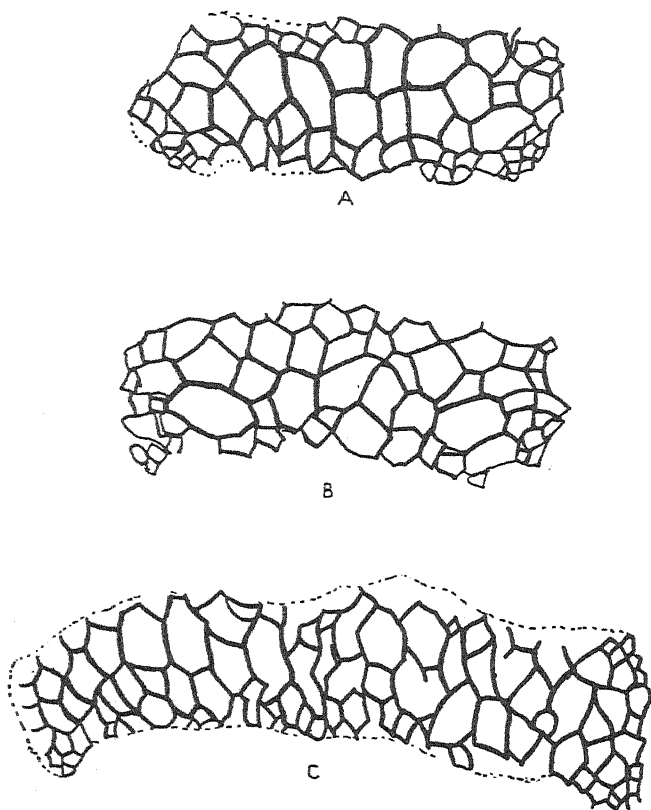
TEXT-FIG. 3. Diagram to show relations of leaf-traces (*lt*) and root-strands (*rt*), the stele (*st*) and the sclerotic nests (*sc*). Based upon the British Museum specimen, but simplified and partly restored.  $\times$  ca. 5.

*loti* (loc. cit., Pl. XXXI, Fig. 1, *bis*). This seems to be the typical condition, but in a few cases one or both these protoxylems appear to form salients projecting adaxially, a fact which may contribute to the curved appearance of some of the bundles. In the strand shown in Text-fig. 4 C, and in a few others, even the metaxylem elements on the adaxial side are on the whole narrower than those on the abaxial.

*Petiole strand.*—It is well known that the permanent form of the bundle is a straight band. Beck mentions the frequent occurrence of bundles with a slight adaxial curve. As he says, these are usually found in petioles which are obviously deformed. The same feature is seen in a few of the curved petioles in the London specimen (Pl. XXXV, Fig. 8 *c. l. t.*). Although the curvature is invariably in the same direction and leads to the suspicion that it is a normal feature comparable to the transient curve seen in *Ankyropteris* and other zygopterids, I am inclined to agree with Beck's view that it is due to strains caused prior to petrification. As already stated, the smaller tracheides frequently tend to lie more towards the adaxial face of the band, and this would make it always curl in the same direction when subjected to desiccation or mechanical strain.

*Pinna-trace.*—Round the petiole trace there is always a dark band of crushed tissue. In some of the petioles this dark zone forms a distinct bulge over one end of the strand (Pl. XXXV, Fig. 1, *p. tr.*). These processes are no doubt due to pinna- (or? aphlebia-) traces given off alternately from

the two margins of the petiolar strand. The incipient pinna-trace is sometimes recognizable as a light-coloured patch in the centre of the little bulging mass of dark tissue, but the structure cannot be made out. I have



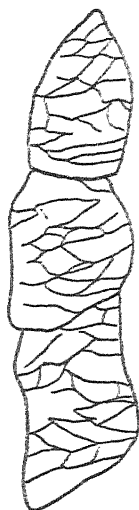
TEXT-FIG. 4. Camera lucida sketches of three foliar bundles from the type-specimen (Freiberg, sec. II). A, B from the outer cortex, C from a free petiole. In B and C (left-hand side) the protoxylem seems to form a salient on the adaxial corner of the margin; but the preservation is imperfect, and on the right-hand side the protoxylem is clearly marginal. In C, which shows a slight curvature, the tracheides along the adaxial face are somewhat narrower than the abaxial. The slide was tilted at angles of about 20 degrees (A and B) or 30 degrees (C) to correct the obliquity of the sections. In all cases the adaxial side is shown facing downwards.  $\times$  ca. 77.

not met with any of these strands farther out in the cortex, nor is there any sign of actual pinnae or aphebiae.

*Ramentum*.—Between the petioles, especially in the peripheral parts of the fossil, there is a dense packing of long and apparently unbranched non-glandular hairs, which in places are clearly seen attached to the epidermis (Pl. XXXV, Figs. 6, 13). The preservation is not good enough to show whether the hairs are unicellular or multicellular.

*Root.*

The relation between the roots and leaves has already been described; no roots have been seen arising from the stem. In the upper parts of the fossil (Pl. XXXV, Figs. 5, 6) very few roots are to be seen between the petioles, although several leaf-traces in the inner cortex show a root-strand arising from either their abaxial side or from one of the margins (Pl. XXXV, Figs. 6, 15). In the root-felt round the base of the stem (London specimen, Pl. XXXV, Figs. 7, 8) most of the roots are seen transversely cut, showing that their general trend (after their initial horizontally outward course) was downward. The stele is usually bipolar and fusiform, a type commonly found in the Osmundaceae and Zygopterideae (Pl. XXXV, Figs. 2, 3). The protoxylem elements are very narrow and often crushed out of recognition. The main part of the stele is formed of very large tracheides; often there is an unusually large central tracheide or two surrounded by smaller ones. In a few (presumably older) roots the outline of the stele is almost circular and there is a faint suggestion of secondary growth (Pl. XXXV, Fig. 4) but the preservation is not quite clear on this point. I have not seen any branching roots. The pitting of the tracheides is of the same kind as in the ordinary tracheides of the stem stele or leaf-trace. The cortex is usually badly crushed. No root-hairs can be made out. I have not found any roots intruded into the petioles or into the stem.



TEXT-FIG. 5.  
A few parenchymatous tracheides from the central xylem (London specimen). The sketch was made from a photograph of a longitudinal fracture of the stele.  $\times$  ca. 186.

*Diagnosis.*

*Grammatopteris Baldaufi* (Beck) Hirmer.

- 1916 *Tubicaulis* sp., Nindel (7, p. 71).
- 1920 *Protothamnopteris Baldaufi*, Beck (1).
- 1927 *Grammatopteris* (*Protothamnopteris*) *Baldaufi* (Beck) Hirmer (5, p. 538).
- 1931 *Grammatopteris Baldaufi*, Hirmer (9, p. 111).

*Diagnosis.* Small tree-ferns with a basal root-felt, and a thick armour of cylindrical petioles 6–8 mm. in diam.; stipules absent. Ramentum hairy, non-glandular. The cylindrical protostele (3.75 to 5 mm. across) is devoid of secondary tissues and consists only of tracheides: a stellate central xylem composed of mixed ordinary and parenchymatous tracheides, and an outer xylem invaginated along the periphery. The protoxylem cannot

be located with certainty, but is probably represented by scattered groups of narrow tracheides in the central xylem. Stem cortex with numerous sclerotic nests. Leaf-traces spiral, at first elliptic, finally assuming the form of a straight tangential band (about 2 mm.) with two marginal protoxylems. Pinna- (or ?aphlebia-) traces minute, in two alternate rows. Free pinnae or aphlebiae not seen. Root-strands diarch, usually devoid of secondary xylem, arising from the abaxial sides of the leaf-traces. Fertile organs unknown.

*Locality.* Two specimens are known, both from Hilbersdorf, a suburb of Chemnitz i. S.

*Age.* Lower Permian (oberer oder Zeisigwalder Porphyrtuff des Mittleren Rotliegenden).<sup>1</sup>

*Specimens and sections.* See Text-figs. 1, 2.

#### COMPARISON WITH *G. Rigolloti*.

(10, pp. 46-7, Pl. XXX, Figs. 9-10, Pl. XXXI, Figs. 1, 1 bis).

In the above description of *G. Baldaufi* two important points in the anatomy have been left rather vague: firstly, the position of the protoxylem in the stem stele, secondly, its position in the foliar bundle. It was to be hoped that a comparison with the contemporary French species<sup>2</sup> would help to clear these points, but unfortunately our knowledge of the latter plant is itself very unsatisfactory. The stele of *G. Rigolloti* is shown in Renault's figures as having a smooth surface, without invaginations. The protoxylem is stated to be exarch, forming definite groups which are said to project from the surface of the stele, but the figures do not confirm this. The xylem is described as a homogeneous tissue, with no differentiation into central and outer regions. If this is confirmed, it would constitute an important point of difference from the German species. The whole structure, however, needs a careful reinvestigation<sup>3</sup> before a satisfactory comparison can be made.

In the petiolar strand, according to Renault, the protoxylems lie definitely at the two extremities, but the condition in the incipient leaf-trace is not described. The cortex is said to be formed of two zones, the inner parenchymatous, the outer sclerenchymatous; but there is no mention of sclerotic nests. Renault does not describe any ramentum, nor does he mention any roots.

<sup>1</sup> See Geologische Spezialkarte von Sachsen, dritte Auflage (1908), p. 31 of the Erläuterungen; also Sterzel (17).

<sup>2</sup> The beds of the Autunian series, to which *G. Rigolloti* no doubt belongs, were once vaguely classed as Permo-Carboniferous, but are now definitely correlated with the Lower Permian (see 16, pp. 247, 251). The Autun beds have yielded a flora closely allied to that of Chemnitz.

<sup>3</sup> Prof. Paul Bertrand kindly showed me at Lille (February, 1930) some thin sections of Renault's type-specimen, but as I only saw these sections cursorily, and before I had carefully examined *G. Baldaufi*, I am limited, in my comparisons, to the published facts.



AFFINITIES OF THE GENUS *GRAMMATOPTERIS*.

At present the genus is usually classed with *Botryopteris* and *Tubicaulis* in the family Botryopterideae,<sup>1</sup> equivalent in rank with the Zygopterideae and Anachoropterideae. But this is admittedly a provisional arrangement. Renault (10) compared the foliar bundle with a zygopterid leaf-trace devoid of the vertical arms. In 1907 Kidston and Gwynne-Vaughan suggested that *Grammatopteris* 'possesses a type of structure that may be regarded as primitively Osmundaceous' (6, Pt. I, p. 778). In 1920 Dr. Scott regarded the genus as 'closely allied to *Botryopteris*, and perhaps forming the simplest type of the family' (13, p. 350). At the same time he tentatively suggested that 'possibly the genera *Grammatopteris* and *Tubicaulis*, *incertae sedis*, may help to bridge the gap' between the Botryopterideae and Zygopterideae; but he cautiously added that 'we are at present without any clear indication of their supposed common ancestry'.

(a) *Relations with the Botryopterideae.* From the description given above it would appear that *Botryopteris* can claim only a remote and indirect affinity with *Grammatopteris*. In the leaf-trace and petiolar bundle there is no resemblance whatever; the phyllotaxis and, therefore the habit, is also quite distinct. In the stem the solid stele is a feature in common, and the pitting is also similar. But the stele of *Botryopteris*, with its centrally placed protoxylem, is much simpler than that of *Grammatopteris*. The resemblances with *Tubicaulis*, on the other hand, are distinctly closer. They consist in the absence of an evanescent mesarch stage in the leaf-trace, which is exarch from the moment it leaves the stele; in the solid character of the stele, and in the presence of narrower tracheides at the periphery, although it is doubtful whether these tracheides in our plant represent the protoxylem. The pitting also resembles that in *T. Berthieri* (2, Pl. III, Fig. 19). But there are also important points of difference, e.g. in the form of the petiolar bundle and, in *G. Baldaufi* at least, in the differentiation of the stele into two zones. In the descriptive part of this paper it was suggested that the two lateral protoxylems of the foliar strand sometimes appear to lie somewhat adaxially, although in the best-preserved cases they are clearly placed along the extreme margins. If this supposed adaxial tendency could be confirmed, even as an occasional feature, the resemblance with *Tubicaulis* would be somewhat enhanced; but the preservation is not decisive on this point.

Viewing the facts as a whole we may say that while there appears to be no real relation with *Botryopteris*, some affinity with *Tubicaulis* may assert itself if certain doubtful points in the anatomy are confirmed.

<sup>1</sup> Seward (15), p. 434; Scott (13), p. 350; Bower (8), p. 10; Gothan (4), p. 122. Hirmer (5), p. 540, places *Tubicaulis* in a separate sub-family Tubicaulidaceae, but groups *Botryopteris* and *Grammatopteris* together in the sub-family Botryopterideae.

(b) *Relations with the Zygopterideae.* The resemblances with the Zygopterideae, on the other hand, are fundamental, although some of them are shared also by the Osmundaceae. The most important zygopterid features are the differentiated protostele with a stellate core<sup>1</sup> and the bipolar, tangentially elongated foliar bundle, symmetrical both in the radial and in the tangential plane.<sup>2</sup> In addition we have the armoured tree-fern habit, with a basal felt of adventitious roots, the hairy ramentum and the association of root- and leaf-traces—features shared also by the Osmundaceae. The absence of 'stipules' would weigh in favour of a zygopterid alliance as against the Osmundaceae.

(c) *Relation with the Osmundaceae.* Perhaps the most important Osmundaceous character is the incisions in the outer xylem which, as already suggested, are probably to be looked upon as rudimentary leaf-gaps. This feature suggests comparison with *Osmundites Dunlopi*, where Kidston and Gwynne-Vaughan described a rather similar condition (6, Pt. I, pp. 760-1), but especially with the condition seen in a newly discovered *Thamnopteris*-like plant which Zalesky (18) has referred to a distinct genus, *Chasmatopteris*. The parenchymatous tracheides in the central xylem recall those of *Chasmatopteris*, *Thamnopteris* and *Zaleskyia*, although in these genera they constituted the whole of the central core; and, as already pointed out, similar elements have been described in *Diplolabis*, and may also have existed in *Asterochlaenopsis* (12, p. 457). The hairy ramentum, the association of root- and leaf-traces, and the tree-fern habit are features common to the Zygopterideae and Osmundaceae. The phyllotaxis, at least in the upper part of the plant, recalls that in *Thamnopteris* and the other Osmundaceae, but the condition in *Asterochlaenopsis* is not very different. Lastly, the presence of sclerotic cells in the stem cortex is perhaps more of an Osmundaceous than a zygopterid feature; but their aggregation into nests is, so far as I know, peculiar to *Grammatopteris*.

(d) *Characters peculiar to Grammatopteris.* If we were to enumerate those features in which this genus stands alone we would mention these sclerotic nests in the cortex, the slightly invaginated protostele and the straight band-shaped foliar bundle, with two marginal protoxylems, but devoid of antennae or peripheral loops.

(e) *Conclusions.* We have seen that the customary grouping of *Grammatopteris* with *Tubicaulis* and *Botryopteris* rests on a slender basis. This was admittedly an artificial arrangement based, as Dr. Scott says, 'on

<sup>1</sup> The composition of the central xylem (mixed parenchymatous and ordinary tracheides) seems very similar to that in *Asterochlaenopsis* (Sahni (12), pp. 456-7, Pl. L, Fig. 10, m, p), but in the latter plant the tracheidal nature of the wider cells is still in doubt. The stele also resembles that of *Diplolabis*, in being devoid of conjunctive parenchyma, but in the latter genus the whole of the central xylem consists of short tracheides.

<sup>2</sup> Unless it is proved that the protoxylems are adaxially situated, in which case the tangential plane of symmetry would be lost.

grounds of convenience, until we are better informed'. *Botryopteris* is an isolated genus and may represent a family of its own; *Tubicaulis* is also perhaps best assigned to a distinct family, as Hirmer has suggested. The affinities of *Grammatopteris* lie clearly in the direction of the Osmundaceae and Zygopterideae: and the main question is to decide, if possible, to which of these families it is most nearly related.

*Grammatopteris* is easily brought into line with the Zygopterideae: the foliar bundle, whether its simplicity is regarded as an original feature or as due to reduction, fits in readily with the zygopterid ground-plan. If the genus is ranged among the zygopterids, it would be geologically one of the youngest; this may fit in with the reduction theory, so far as the foliar bundle is concerned, but the stele is of a very primitive type, with only a few of the central tracheides modified for water storage. The structure as a whole seems more consistent with the alternative view, that *Grammatopteris* is a primitive type of zygopterid, which has persisted till the Permian, most of its allies being older.

If, on the other hand, *Grammatopteris* is placed among the Osmundaceae, it would figure as a welcome prototype of that family, being not only simpler in structure but also distinctly older than both *Thamnopteris* and *Zalasskya*. Such a course would be supported chiefly by the stem anatomy. The name *Protothamnopteris* invented by Beck would then be particularly appropriate, although we would still have to reject it on grounds of priority. Should *Thamnopteris* and *Zalasskya* ultimately have to be transferred to a special group, a possibility hinted by Kidston and Gwynne-Vaughan (6, Pt. IV, pp. 466-7), *Grammatopteris* would probably have to accompany them, as a primitive member of a family probably directly ancestral to the Mesozoic Osmundaceae.

Thus, while in its foliar characters *Grammatopteris* clearly approaches the Zygopterideae, its stem structure is paralleled by members of both the Osmundaceae and the Zygopterideae. On the whole there seems to me to be a somewhat stronger case for regarding the genus as a zygopterid than for referring it to the Osmundaceae. Professor Seward, who has very kindly criticized the manuscript, writes: 'I cannot help feeling that the affinities are rather more on the side of the Osmundaceae'. I confess the resemblances with that family are very impressive, and the balance is so nearly even that my choice may have been largely determined by the personal factor. But the main fact is that we cannot, with full confidence, assign the genus to either of these families, and this is perhaps the strongest proof of their affinity and their common ancestry.

#### SUMMARY.

This paper is based on a reinvestigation of the type-specimen of *Protothamnopteris Baldaufi*, Beck, a silicified tree-fern from the Lower Permian

of Saxony. Hirmer's reference of the species to the genus *Grammatopteris* is confirmed, and the affinities of the latter genus are discussed.

Reasons are given for the view that *Grammatopteris* should be removed from the Botryopterideae: the structure clearly shows that the affinities lie in the direction of the Zygopterideae and the Osmundaceae. In its stem anatomy *G. Baldaufi* resembles certain members of both these families, but in its foliar characters it readily falls into line with the Zygopterideae. On the whole, so far as the vegetative characters can serve as a guide, the affinity with the latter family appears to the author to be somewhat the stronger.

*Grammatopteris* is a synthetic type of great interest and affords an important piece of evidence in favour of Kidston and Gwynne-Vaughan's theory of a common origin for the Osmundaceae and Zygopterideae. That this genus is both simpler in structure and geologically older than the allied genera *Thamnopteris* and *Zaleskya*, probably indicates that it is not far removed from the primitive Osmundaceous stock. At the same time the simple bipolar leaf strand, as well as the stelar structure, probably mark it out as a primitive type of zygopterid.

The discovery of *Grammatopteris* in the Lower Permian beds of Chemnitz provides a further link with the allied flora of Autun in Central France, which is roughly contemporaneous with that of Saxony.

It is a pleasant duty to express my sincere thanks to all those named above, and also to Dr. E. Strauss of Chemnitz, Professor W. Gothan and Dr. J. Schuster of Berlin, Dr. J. Kisser of Vienna, and to Professor Schreiter and Dr. K. A. Jurasky of Freiberg i.S. for the information, material, and laboratory facilities they so kindly gave me during my tours. As on previous occasions, I am deeply indebted to Professor A. C. Seward for valuable criticisms made during a revision of the manuscript.

LUCKNOW, INDIA,

April 20, 1932.

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## EXPLANATION OF PLATE XXXV.

Illustrating Professor Sahni's paper On a Palaeozoic Tree-fern, *Grammatopteris Baldaufi* (Beck) Hirmer, a link between the Zygopterideae and Osmundaceae.

(Abbreviations: *a.l.t.*, adaxially curved leaf-trace; *i.c.*, *m.c.*, *o.c.*, inner, middle, and outer cortex; *i.l.t.*, incipient leaf-trace; *inv.*, invaginations in the outer xylem; *par.tr.*, parenchymatous tracheide; *p.tr.*, position of pinna-trace; *p.x.*, protoxylem; *r.*, root; *ram.*, ramental hairs; *r.t.*, root-trace; *sch.*, sclerotic zone; 1 x<sub>2</sub> doubtful secondary xylem.)

(All the figures are from untouched photographs).

Fig. 1. Freiberg specimen, sec. II (cf. Text-fig. 1). The petiolar strand at \* is enlarged in Fig. 16. × 3.2.

Figs. 2, 3. London specimen. Diarch steles of two roots from the root-felt (section at 24 mm.; see Text-fig. 2). × ca. 55.

Fig. 4. London specimen, same section. Stele of an older root, with 2 secondary xylem. × ca. 55.

Fig. 5. Freiberg specimen, sec. II, showing details of stele; cf. Fig. 10. × ca. 18.

Fig. 6. Freiberg specimen, sec. I, \*\* outline of stem with decurrent leaf-bases, marked with Indian ink. × 2.6.

Fig. 7. London specimen, section at 24 mm. (see Text-fig. 2). Nat. size.

Fig. 8. Part of the same section. × ca. 3.5.

Fig. 9. Part of the same section to show the phyllotaxis. The stellate central xylem is clearly seen. × ca. 4.7.

Fig. 10. The same section showing details of stele; cf. Fig. 5. × ca. 18.

Fig. 11. London specimen, part of longitudinal section at c (Text-fig. 2) passing tangentially through the outer xylem. × 133.7.

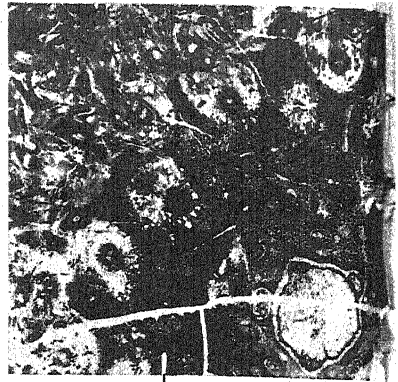
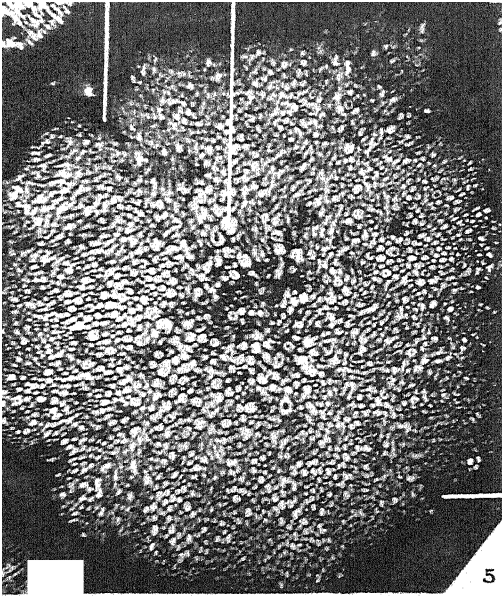
Fig. 12. London specimen. Part of a sclerotic nest from section at 24 mm. Note the pit-canal. × ca. 60.

Fig. 13. Freiberg specimen, sec. II. Surface of a petiole covered with a dense ramentum of hairs. × 15.5.

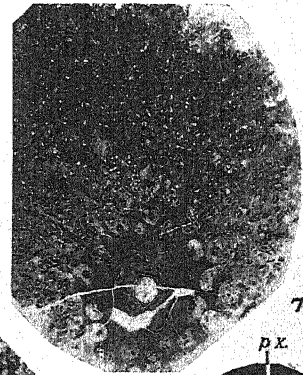
Fig. 14. Freiberg specimen, sec. I. A leaf-trace from the inner cortex, resembling a root-stele. The slide was tilted at an angle of 20 degrees under the camera to correct the obliquity of the section. × ca. 40.

Fig. 15. Same section. A leaf-trace in the inner cortex with a root-strand coming off from its abaxial side. The slide was tilted at 45 degrees for correction. × ca. 45.

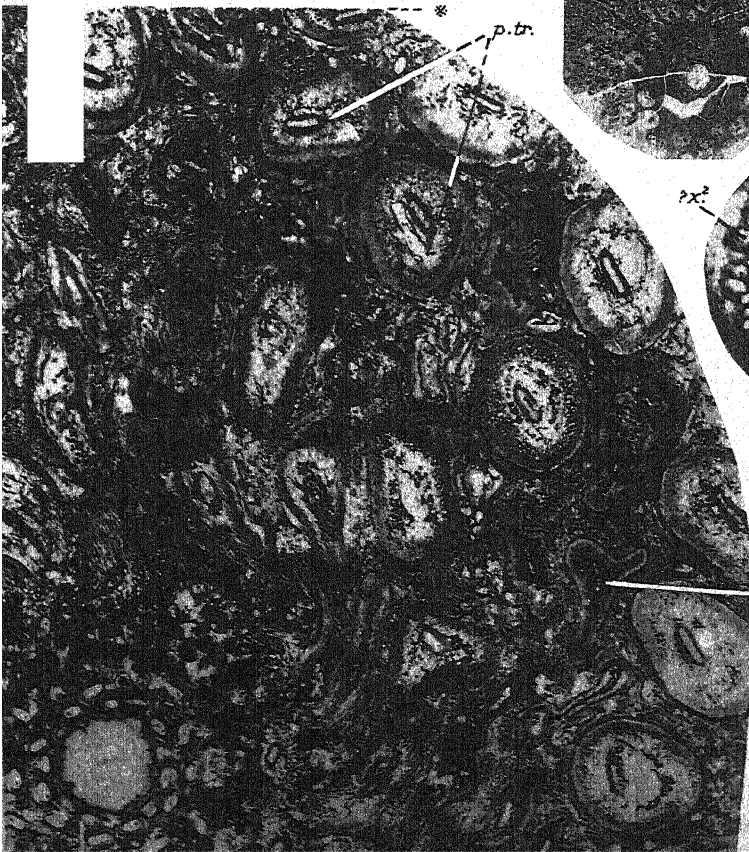
Fig. 16. Freiberg specimen, sec. II. Petiolar strand of leaf marked \* in Fig. 1. The slide was tilted at 45 degrees for correction. × ca. 42.



*scl. z.*



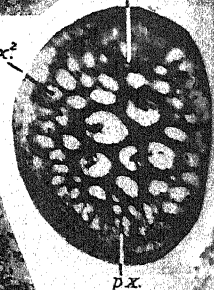
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*p. tr.*

*inv.*

5



*p. x.*

*p. x.<sup>2</sup>*

*p. x.*

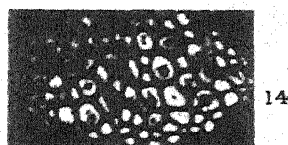
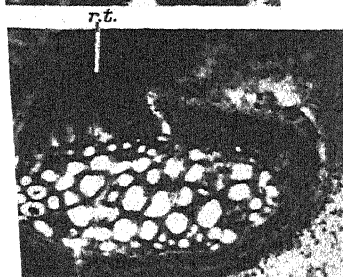
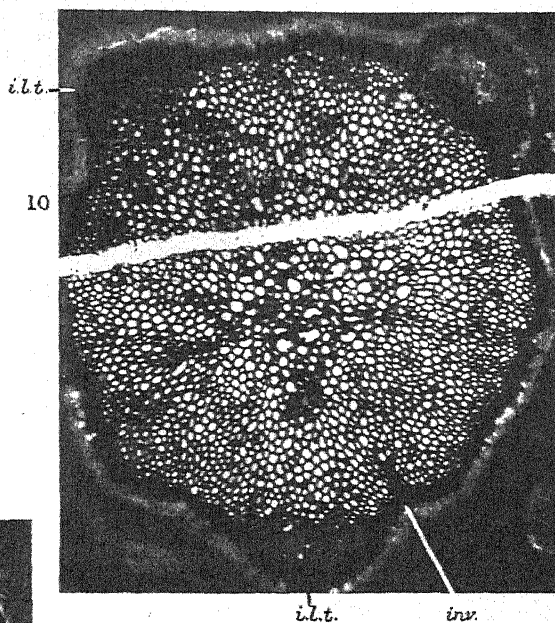
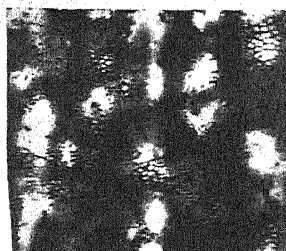
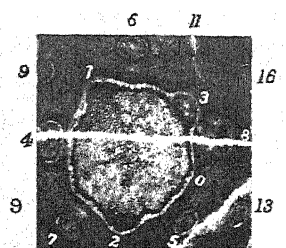


*c. l. t.*

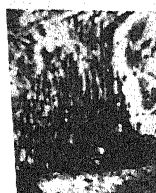
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15



6





# Effect of Plane-polarized Light on the Formation of Carbohydrates in Leaves.

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With Plate XXXVI.

## INTRODUCTION.

THE biological effects of radiations like the X-rays, the ultra-violet, and the radium emanations have been known for some time, but it is only recently that the effect of polarized radiations on chemical and biological processes has formed the subject of scientific investigation. Baly and Semmens (1 and 2) were perhaps the first to study the action of the polarized light on the hydrolysis of starch. They found that the plane-polarized light accelerated the hydrolysis of starch.

Macht and his collaborators (15, 16, 17, 18, 19, 20, 21) have also studied the effect of the polarized light on the pharmacological properties of several drugs, on the fermentive power of enzymes like rennin and catalase, on rats injected with convulsant drugs like cocaine, santonin, and camphor, and on the growth of yeast and bacteria. In each case they found some noticeable effect of the polarized light.

The effect of the polarized light on certain photochemical reactions and the growth of bacteria was studied in India by Bhatnagar and his associates (3, 4, 5, 6). They also lent support to the conclusion arrived at by Baly and Semmens (1 and 2) and Macht and his associates (15-21) that there was a specific effect of the polarized light on some chemical reactions and the growth of micro-organisms.

A contradictory view, however, was expressed by Jones (13) and Bunker and Anderson (7), who were unable to repeat the observations of the above-mentioned investigators.

The most conclusive evidence against the view of Baly and Semmens was obtained by Navez, Albert, and Rubenstein (22). They have investigated the problem of starch hydrolysis under very carefully controlled and uniform conditions. They thought that little attention was paid by the previous workers to the properties of light that emerged from the pile of polarizing plates, and no indication was given of the type of enzyme used. They arrived at the following conclusions about the starch-diestase system from their investigations:

(1) 'Ordinary light and polarized light of the same intensity, and as closely as possible similar in spectral composition, have the same effect.'

(2) 'Light increases the rate of hydrolysis as compared to the dark.'

Macht (15) also studied the effect of polarized light on the growth of higher plants. He measured the increase in length of lupine seedlings in polarized and non-polarized radiations and found that the roots grew better in the former. Similar experiments were made with squash, wheat, and sunflower seedlings. The experiments on the wheat seedlings were of interest as the weights of the seedlings exposed to the two kinds of light were also taken, although the results were not included in the paper. It is now generally accepted that the more satisfactory method of studying the process of growth is to measure increase in weight rather than the increase in length. All the results of Macht are given in length measurements; moreover, the differences obtained in the lengths of the seedlings are very small. The lamp used by Macht (15) was of 700 c.p. The light emerging from the pile of glass plates must have considerably lost in intensity; possibly this may have acted as a limiting factor, and therefore the irradiated seedlings showed signs of etiolation. The greater increase in the lengths of the seedlings in polarized and non-polarized light as compared with their lengths under normal conditions supports the above idea. If the seedlings are etiolated the results obtained by Macht are open to criticism.

Semmens (25) has recently obtained further evidence in support of her views concerning the action of polarized light on the hydrolysis of starch in the living leaf. She has shown that starch disappears more rapidly from the leaf area exposed to polarized light than from that kept in the dark. She has also shown that a leaf exposed to polarized light from the blue sky is denuded of starch. She concludes from her observations that starch is formed in the non-polarized daylight and is hydrolysed in the polarized light of the late afternoon. She has also tried the effect of polarized light upon the growth of the whole plant, and has found that it suffered in vigour and growth as no starch was produced under the polarized radiations.

Crozier and Mangelsdorf (8) also studied the effect of polarized light on tropistic reactions. They obtained, however, no significant differences between responses to ordinary and polarized light.

The above review of work on the effect of the polarized light on chemical reactions and biological processes shows that opinion is sharply divided on the subject. The experiments of Macht (15) on the growth of seedlings, and those of Semmens (25) on the hydrolysis of starch in a living leaf are not very convincing, and it is premature to affirm that polarized light has any specific effect on these two processes.

It has been suggested that the polarization of sunlight may be one of the asymmetric forces outside the plant responsible for the formation of optically active products in the photosynthetic process, although the manner in which it affects chemical reactions, if at all, is not known. In spite of repeated and careful attempts to effect a direct asymmetric synthesis by means of physically asymmetric influences, no success has been attained.

In order to test the validity of the above suggestion it seemed worth while in the first instance to study the effect of the polarized radiation on the rate of photosynthesis as compared to that of the non-polarized one. Will there be any increased production of optically active substances in the leaf if the percentage of the polarized radiation is increased? As, so far as the writers are aware, nobody has yet studied the effect of polarized light on this important process, the present investigation was undertaken. According to Semmens (25) the sunlight from a blue sky is more or less polarized during the afternoon, and it brings about a rapid hydrolysis of the starch produced in the ordinary light. She has also shown that plants are not able to produce starch in the polarized light, and as a result they show signs of starvation and death. These are very important conclusions in relation to the fundamental problem of photosynthesis, and an attempt to test their validity by careful experimentation would seem to be of value.

In the investigation plane-polarized light is used. The plane-polarized light could be obtained in two ways—by passing light through a nicol prism, or by means of a pile of glass plates kept at the angle of maximum polarization to the incident beam of light. The use of a nicol prism to obtain a beam of polarized light was out of question in this investigation since the beam obtained would have been too small to illuminate the whole plant. The glass pile method was, therefore, used in this investigation.

#### EXPERIMENTAL.

It may not be out of place briefly to recall several unsuccessful attempts made in this investigation, as a number of difficulties had to be overcome in measuring the rate of photosynthesis, as well as in illuminating the whole plant with a sufficiently large and highly polarized beam of sufficient intensity.

At first an attempt was made to study the effect of polarized light on photosynthesis by collecting the gas bubbles given out by *Hydrilla viridis*, a water plant, under constant conditions as regards temperature, light intensity, &c., and by making a quantitative analysis of the various components of the gas-mixture. The plant was, however, found to show a very erratic and irregular behaviour, the gas-mixture collected in four hours being at times hardly 0.01 c.c., and at times nil. This procedure had, therefore, to be abandoned.

On account of this unforeseen difficulty with water plants it was then decided to study the effect of polarized light on land plants. It was thought advisable to study the various carbohydrates formed in the two kinds of light rather than estimate the amount of carbon dioxide absorbed. An improved colometric method of estimating the various carbohydrates, developed by Dastur and Samant (9), was adopted. Also a new plan of exposing the plant to polarized light was devised.

Macht and Anderson (15) have devised a very simple and convenient arrangement for studying the effect of polarized light on seedlings. Following the plan of their apparatus a bigger and more roomy box was constructed with necessary changes to suit the needs of this investigation. The box was of the shape of a truncated pyramid with the back wall perpendicular to the base and the three other sides inclined. The top of the box was kept open, and on it was placed a water screen *w* to cut off the heat rays from the overhanging lamp. The lamp used was a 1500 watt, gas-filled, 230-volt Phillips bulb. The whole arrangement is shown in Pl. XXXVI, Fig. 1.

The number of plates used for polarizing the light was twelve. The intensity was equalized in the two cases by adjusting the number of plates in the ordinary light. The intensity measurements were made by using a microthermopile (Cambridge Science Inst. Co.), along with a D'Arsonval galvanometer (Tinsley & Co.).

As one plant only could be properly illuminated in the chamber, the following procedure was adopted in every experiment.

From a group of eighteen plants, of nearly the same age and previous history, batches of three plants were put in the dark at the interval of four hours. On the third day in the morning, i.e. forty-eight hours later, the first batch was used for the experiment. Two plants were exposed, one to each kind of light, and the third was taken directly for carbohydrate analysis. In the afternoon the second batch was utilized. This procedure was repeated for three successive days, and the leaf-extracts obtained of three identical experiments were eventually mixed so that a fairly average result could be obtained. Thus in each case leaves from half a dozen plants were picked, and the results represent an average of about two dozen leaves in each case. The method of extracting the carbo-

hydrates from the leaves, worked out by Davis, Daish, and Sawyer (10, 11, 12) was adopted in this investigation.

# RESULTS.

The results given in Table I and those presented later are expressed as per 100 gm. of fresh leaves.

TABLE I.

*Abutilon asiaticum*, G. Don., 24. 7. 30 to 26. 7. 30.

	Total sugars as hexoses.	Starch as hexoses.	Total carbohydrates as hexoses.
	gm.	gm.	gm.
Dark . . . . .	0.078	0.055	0.133
Non-polarized light . . . . .	0.100	0.051	0.151
Polarized light . . . . .	0.084	0.044	0.128

*Allium cepa*, L., 15. 8. 30 to 17. 8. 30.

	Hexoses.	Sucrose as hexoses.	Total sugars as hexoses.
	gm.	gm.	gm.
Dark . . . . .	0.1015	0.1831	0.2846
Non-polarized light . . . . .	0.0754	0.1399	0.2163
Polarized light . . . . .	0.0635	0.1640	0.2275

*Allium cepa*, L., 20. 8. 30 to 22. 8. 30.

	Total sugars as hexoses.
	gm.
Dark . . . . .	0.3455
Non-polarized light . . . . .	0.3334
Polarized light . . . . .	0.3228

It was realized from these results that photosynthesis did not go on normally in these experiments. Evidently the only factor limiting it was light intensity. It was thought advisable, therefore, to raise the light intensity. It was not possible to do so with the lamp used, as the whole arrangement set up was just enough to give the maximum intensity of light. It was decided, therefore, to substitute a floodlight lamp, and one of Novalux Projector type with a 1000 watt bulb, manufactured by the General Electric Co., Shenectady, N.Y., U.S.A., was obtained. The use of the floodlight lamp introduced, however, another serious difficulty. Its bulb could burn either in a horizontal position or with the base pointing downwards. The only course left open was to work with reflected polarized light instead of transmitted. Accordingly another arrangement had to be made.

A big wooden frame (Pl. XXXVI, Fig. 2), accommodating about two dozen glass plates, 24 in. by 24 in. in size, was set between two strong wooden poles, fixed to a strong, firm wooden base, in such a way that it could easily be rotated in the vertical plane. The plates (twenty in number) were set at the angle of  $57^\circ$  to the incident beam of light. The light that was reflected down from the plates was thus highly polarized. The arrangement is shown in Pl. XXXVI, Fig. 2. Dark screens used to cut off any direct or reflected rays other than the polarized beam are not shown in the photograph.

Non-polarized light was obtained from an ordinary 1500 watt gas-filled lamp. Its intensity was matched with that of the polarized light by adjusting the distance between the bulb and the plants. It may be argued that these two sources of light may not be identical as regards their spectral intensities, and if it were so the results obtained would not consequently be comparable. A comparative study of the spectral intensities of two equally intense beams from the two sources was, therefore, made with the help of various glass filters of the Wratten 'M' series manufactured by Kodak & Co. Each filter was placed over the micro-thermopile, and the deflexions of the galvanometer were noted in both the beams. Two sets of readings were taken, and it was found that there were no differences in spectral intensities in the two sources of light.

The results of the experiments are given in Table II. As the process of extracting and estimating the various carbohydrates is very lengthy, the number of experiments is limited. Each set of experiments took about six weeks to complete.

The results of the five sets of experiments do not show any accelerating or retarding effect of the polarized light on the formation of carbohydrates in the leaves of *Allium cepa*, L. If the total sugars obtained in the five sets of experiments in the two cases are added up we get 3.973 grm. per 500 grm. of fresh leaves in the non-polarized light, and 3.998 grm. in the polarized light.

Whether the differences in the carbohydrates formed in the two lights are significant or not can be statistically examined by Fisher's method of  $t$  adapted to biological work involving a small number of experiments.  $t$  is determined by the formula

$$\frac{\text{Mean}}{\sqrt{\frac{\sum (d)^2}{n(n+1)}}}$$

these experiments is equal to 0.0348. Then from Fisher's table,  $P$  to or greater than 0.9 when  $n = 4$ . Therefore the differences are statistically significant.

was then decided to repeat the experiments with starch-forming

leaves as the leaves of *A. cepa*, L., are known to produce sugars only in photosynthesis. A series of experiments was, therefore, performed with the giant and the small-leaf varieties of *Helianthus annuus*, L. Leaves from ten plants were taken for carbohydrate analysis in each case.

TABLE II.

Experiment (1). *Allium cepa*, L., 17. 12. 30 to 18. 12. 30.

	Reducing sugar.	Sucrose as hexoses.	Total sugars as hexoses.
	gram.	gram.	gram.
Dark . . . . .	0.339	0.131	0.470
Non-polarized light . . . . .	0.395	0.126	0.521
Polarized light . . . . .	0.421	0.051	0.472

Experiment (2). *Allium cepa*, L., 30. 12. 30 to 31. 12. 30.

Dark . . . . .	0.615	0.218	0.833
Non-polarized light . . . . .	0.635	0.250	0.885
Polarized light . . . . .	0.655	0.290	0.945

Experiment (3). *Allium cepa*, L., 8. 1. 31 to 9. 1. 31.

Dark . . . . .	—	—	—
Non-polarized light . . . . .	0.693	0.195	0.888
Polarized light . . . . .	0.472	0.122	0.864

Experiment (4). *Allium cepa*, L., 15. 1. 31 to 17. 1. 31.

Dark . . . . .	0.484	0.231	0.715
Non-polarized light . . . . .	0.574	0.279	0.853
Polarized light . . . . .	0.521	0.257	0.778

Experiment (5). *Allium cepa*, L., 22. 1. 32 to 24. 1. 31.

Dark . . . . .	0.711	0.142	0.853
Non-polarized light . . . . .	0.462	0.364	0.826
Polarized light . . . . .	0.770	0.169	0.939

In both sets of experiments starch was formed as well in the polarized light as in the ordinary one. The results did not support the view of Semmens that starch is hydrolysed in the polarized light and is never formed in it.

It would appear from the results given above that the polarized light has neither an accelerating nor a depressing effect on the rate of photosynthesis. The light used in these experiments was artificial. In view of the recent work on the formation of carbohydrates in leaves exposed to artificial light and diffused sunlight by Dastur and Samant (9), who have shown that the amount of carbohydrates produced in the artificial light of the same or even higher intensity is much less than that produced in the diffused sunlight during the same period, it would be necessary to perform these experiments in sunlight in order to determine whether polarized sunlight has any effect on photosynthesis as compared with the

non-polarized sunlight of the same intensity. Experimentation with polarized sunlight brings up a number of difficulties. The source of light in this case was changing its position every minute and the rays therefore struck the glass plates at different angles. The difficulty could have been met by the use of a heliostat, but the heliostats available had mirrors too small to illuminate even a single plant.

TABLE III.

Experiment (1). *Helianthus annuus*, L. Small-leaf variety 11. 2. 31 to 13. 2. 31.

	Reducing sugars as hexoses.	Sucrose as hexoses.	Starch as hexoses.	Total carbohydrates as hexoses.
	gram.	gram.	gram.	gram.
Dark . . . . .	0.009	0.084	0.044	0.137
Non-polarized light	0.019	0.193	0.113	0.325
Polarized light . .	0.011	0.175	0.129	0.315

Experiment (2). *Helianthus annuus*, L. Small-leaf variety 24. 2. 31 to 26. 2. 31.

	Reducing sugars as hexoses.	Sucrose as hexoses.	Starch as hexoses.	Total carbohydrates as hexoses.
	gram.	gram.	gram.	gram.
Dark . . . . .	0.007	0.029	0.086	0.122
Non-polarized light	0.006	0.082	0.075	0.163
Polarized light . .	0.009	0.086	0.124	0.219

It is, however, possible to work without the use of a heliostat if one selects for the experiment a short period of the day when the sun shifts its position practically in one plane. Such a period is noon-time when the sun is at its zenith. The maximum time at disposal would be about two hours—one hour before and one hour after the sun crosses the meridian. During one hour the sun shifts its position by about  $15^\circ$ . During the period selected the sun would, therefore, make an angle of  $15^\circ$  with the meridian on its east and west sides. It would thus pass along a very small arc of a circle during this period. So if a pile of plates is set up so as to make an angle of  $33^\circ$  with the meridian point, the sun-rays coming from that point would make an angle of  $57^\circ$  with the normal to the plates. Thus one hour before noon the angle of incidence would be about  $42^\circ$ . Gradually the angle of maximum polarization would be reached towards noon and then it would again diminish to  $42^\circ$ , during the next hour.

There are, however, several details which should be taken into account. They are, for example, the inclination of the sun which varies with the equinoxes, and the exact time when the sun comes to the zenith. These details were kindly furnished by the Director of the Colaba Observatory, Bombay, and the pile of plates was arranged accordingly (Pl. XXXVI, Fig. 3). By the side of the apparatus was set up another for working with ordinary light (Pl. XXXVI, Fig. 4). In this case the pile of plates for equalizing the light intensity was set at right angles to the path of the sun-



rays. As in the case of the polarized sunlight the sun-rays in the beginning of the first hour would make an angle of  $15^\circ$  with the normal. As the sun approached the meridian the angle would diminish and again increase to  $15^\circ$  at the end of the second hour.

Another difficulty arises in these experiments. There is a certain amount of polarized radiation in sunlight. The percentage of polarization varies from day to day and hour to hour. It depends upon the clouds and the dust particles in the atmosphere. A few measurements were therefore made by means of Babinet's compensator, according to the method briefly described below.

The polarization in the beam of sunlight emerging from the pile of twenty glass plates in the apparatus described above was compensated by nine small and inclined glass plates 3 in. by 3 in. which were placed between the polarized source of light and the compensator. The glass plates were rotated until the central dark bands seen in the compensator became faint, i.e. when the polarization produced by transmission through the small inclined plates exactly compensate the opposite polarization in the beam of sunlight emerging through the pile of twenty glass plates.

If the angle of incidence upon the inclined plates is  $\theta$  and the refractive index of glass is  $\mu$ , the angle of refraction  $\phi$  can be derived from the equation  $\frac{\sin \theta}{\sin \phi} = \mu$ . If  $n$  is the number of plates used for compensation

the intensity ratio is given by  $\cos^4(\theta - \phi)^n$ . If  $\cos^4(\theta - \phi)^n = \frac{1}{x}$  the per-

centage of polarization =  $\frac{x-1}{x+1} \times 100$ . The percentage of polarization in the above experiments was 76.6, 88.6 and 81.6 for the angles of incidence  $47^\circ$ ,  $54^\circ$  and  $50^\circ$  respectively, between 12 noon and 1.15 p.m.

In the apparatus for treating plates with non-polarized light the percentage of polarized light was 18 at 1.30 p.m. which represented the maximum quantity, as the sun had moved away from the meridian point and the rays struck the plates at an angle of  $15^\circ$  instead of at a right angle.

## RESULTS.

TABLE IV.

Experiment (1) *Helianthus annuus*, L. Small-leaved variety, 30. 3. 31 to 31. 3. 31.

	Total sugars as hexoses.	Starch as hexoses.	Total carbohydrates as hexoses.
	gm.	gm.	gm.
Dark . . . . .	0.053	0.049	0.102
Non-polarized light . . . . .	0.193	0.010	0.203
Polarized light . . . . .	0.153	0.082	0.235

TABLE IV (continued).

Experiment (2) *Helianthus annuus*, L. Giant variety, 16. 4. 31 to 17. 4. 31.

	Reducing sugars as hexoses.	Sucrose as hexoses.	Starch as hexoses.	Total carbohydrates as hexoses.
	gram.	gram.	gram.	gram.
Dark . . . . .	—	0.014	0.030	0.044
Non-polarized light . . . . .	—	0.021	0.008	0.029
Polarized light . . . . .	—	0.020	0.021	0.041

Experiment (3) *Helianthus annuus*, L. Giant variety, 20. 4. 31 to 21. 4. 31.

Dark . . . . .	—	0.032	0.076	0.108
Non-polarized light . . . . .	0.002	0.108	0.085	0.195
Polarized light . . . . .	0.002	0.080	0.047	0.129

Experiment (4) *Helianthus annuus*, L. Small-leaf variety, 27. 4. 31 to 28. 4. 31.

Dark . . . . .	0.006	0.049	0.094	0.149
Non-polarized light . . . . .	0.003	0.061	0.195	0.259
Polarized light . . . . .	0.005	0.087	0.179	0.272

When the results are examined statistically  $t = 0.3037$  and when  $n = 5$ ,  $P = > 0.7$ . Therefore the results are not significant.

Another series of experiments was carried out with Radish (*Raphanus sativus*) and the results are given below.

TABLE V.

Experiment (1). 23. 12. 31 to 24. 12. 31.

	Total sugars as hexoses.	Starch as hexoses.	Total carbohydrates as hexoses.
	gram.	gram.	gram.
Polarized . . . . .	0.094	0.019	0.113
Non-polarized light . . . . .	0.115	0.026	0.141
Dark . . . . .	0.035	0.018	0.053

Experiment (2). 15. 1. 32 to 16. 1. 32.

Polarized light . . . . .	0.051	0.032	0.083
Non-polarized light . . . . .	0.043	0.028	0.071
Dark . . . . .	0.035	0.026	0.061

Experiment (3). 22. 1. 32 to 23. 1. 32.

Polarized light . . . . .	0.060	0.055	0.115
Non-polarized light . . . . .	0.120	0.044	0.164
Dark . . . . .	—	—	—

Experiment (4). 10. 2. 32 to 11. 2. 32.

Polarized light . . . . .	0.129	0.046	0.175
Non-polarized light . . . . .	0.289	0.108	0.397
Dark . . . . .	0.093	0.062	0.155

TABLE V (continued).

	Total sugars as hexoses.	Starch as hexoses.	Total carbohydrates as hexoses.
	gram.	gram.	gram.
Experiment (5). 19. 2. 32 to 20. 2. 32.			
Polarized light . . . . .	0.120	0.052	0.172
Non-polarized light . . . . .	0.109	0.026	0.135
Dark . . . . .	0.028	0.013	0.041
Experiment (6). 1. 3. 32 to 2. 3. 32.			
Polarized light . . . . .	0.089	0.012	0.101
Non-polarized light . . . . .	0.043	0.009	0.052
Dark . . . . .	0.028	0.012	0.040

In these experiments  $t = 0.8313$  and when  $n = 5$   $P$  is between 0.5 and 0.4 and therefore the differences are not significant.

#### CONCLUSIONS.

The results obtained were against the view advanced by Semmens of the action of polarized light on starch hydrolysis and ultimately on photosynthesis. According to her view formation of starch ceases in polarized light and the plants exposed to polarized light suffer in vigour. In our results, there was no indication of the destruction of starch in polarized light. The results, on the other hand, indicated that the process of photosynthesis goes on as vigorously and regularly in polarized light as in ordinary light.

#### SUMMARY.

In recent years the study of the effect of polarized light on chemical reactions and biological processes has engaged considerably the attention of scientific investigators. An attempt was, therefore, made to study the effect of polarized light on the formation of carbohydrates in living leaves.

The arrangement of the apparatus and the attainment of a sufficiently large and intense beam of strongly polarized light presented the chief difficulty. Ultimately a floodlight lamp was used to obtain a large and intense beam of reflected polarized light.

A series of experiments was performed with the leaves of *A. cepa*, L., and *Helianthus annuus*, L. The amount of total carbohydrates formed in leaves in polarized light was very similar to that formed in the non-polarized light. On examining the results statistically the differences are found to be of no significance.

Similar experiments were performed in sunlight. The arrangement of the apparatus is described. The results obtained with *H. annuus*, L., and

*Raphanus sativus* were similar to those obtained in artificial light. The results showed that in polarized light photosynthesis goes on as well as in ordinary light. The formation of starch was found to occur in polarized light, which is contradictory to the conclusions drawn by Semmens (25).

## EXPLANATION OF PLATE XXXVI.

Illustrating Professor Dastur and Mr. R. D. Asana's paper on The Effect of Plane-polarized Light on the Formation of Carbohydrates in Leaves.

Fig. 1. Arrangement of the apparatus for exposing plants to transmitted polarized and non-polarized lights from an electric light.

Fig. 2. Arrangement of the apparatus for exposing plants to reflected polarized light from a flood-light lamp.

Fig. 3. Arrangement of the apparatus for exposing plants to transmitted polarized sunlight.

Fig. 4. Arrangement of the apparatus for exposing plants to non-polarized sunlight.

In all figures P. = polarized; N. = non-polarized; W. = water screen; L. = lamp; F. = wooden frame holding a pile of glass plates.

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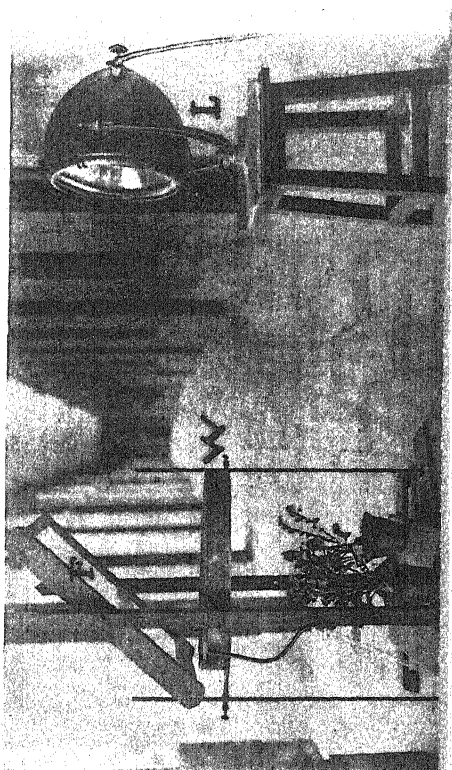
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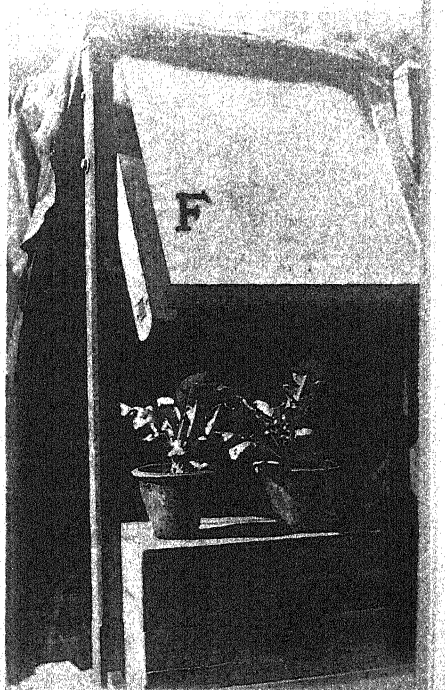
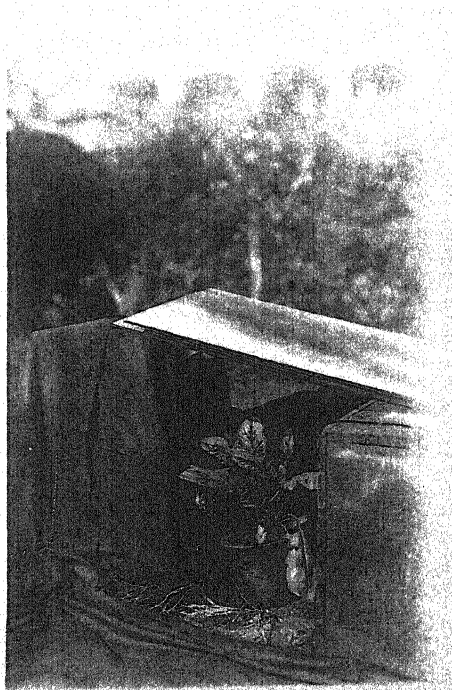




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2







# An Investigation by Cultural Methods of some of the Factors Influencing the Development of the Gametophytes and the Early Stages of the Sporophytes of *Laminaria digitata*, *L. saccharina*, and *L. Cloustoni*.

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With Plates XXXVII-XXXIX and thirteen Figures in the Text.

A DEFINITE rhythm has been observed in the life-cycle of the three common species of *Laminaria*, namely, *L. digitata*, *L. saccharina*, and *L. Cloustoni*. Reproduction takes place from August to March, and in all species, towards the end of the reproductive period, growth recommences in the region of junction between the thallus and the stipe. The formation of the new thallus proceeds rapidly during spring and early summer, but, in all species, growth of the thallus is negligible for a few months in the year ranging from June to January.

From September 1929 to September 1930, the first appearance of zoosporangia was recorded for the three species, and counts of from fifty to a hundred plants were made whenever the tides were suitable. The percentages of the plants bearing mature fertile areas are given in Table I A and Text-fig. 1 A. Data concerning growth were also collected, the length of the new thallus being, in each case, measured from the top of the stipe to the constriction at the base of the thallus of the previous year. When time permitted, the greatest width of the thallus was noted. The average lengths and breadths of fifty to a hundred thalli of each species are recorded in Table I B and Text-fig. 1 B. A comparison of Text-figs. 1 A and 1 B will show the correlation between the time of reproduction and growth.

The order of maturation and of vegetative growth of the three species can also be correlated with their zonation on the shore, that is, first *L. digitata*, then *L. saccharina*, and finally *L. Cloustoni*.

It has been observed that the times of reproduction and growth vary in different years, but a definite periodicity is always evident and would appear to be dependent upon mineral content of the sea-water, temperature, and light intensity.

TABLE I A.

Date.	<i>Laminaria digitata.</i>	<i>Laminaria saccharina.</i>	<i>Laminaria Cloustoni.</i>
Sept. 18, 1929	Beginning		
Oct. 19, "	Many		
" 21, "	80 %		
Nov. 4, "	100 %	Occasional	
Dec. 2, "	50 %	80 %	
" 16, "	20 %	100 %	Beginning
" 31, "	Nearly over	100 %	
Jan. 3, 1930			100 %
" 15, "		50 %	
" 18, "		40 %	14.2 %
Feb. 1, "		31.5 %	Nearly over
" 13, "		24 %	
" 17, "		10 %	
Mar. 4, "		Nearly over	

Percentages of thalli of *Laminaria* bearing mature zoosporangia.

TABLE I B.

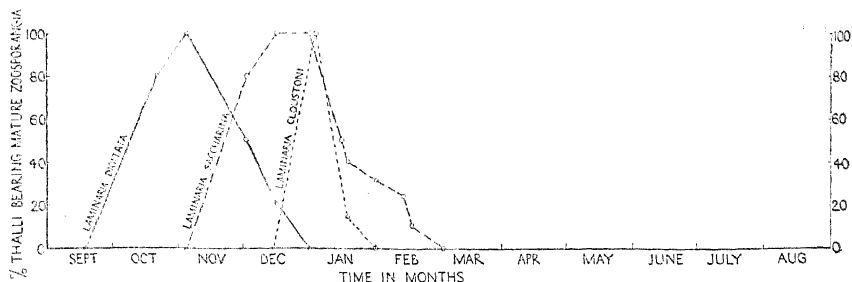
Date.	<i>Laminaria digitata.</i>	<i>Laminaria saccharina.</i>	<i>Laminaria Cloustoni.</i>
Dec. 16, 1929	Beginning		
" 31, "	2.5 in.		
Jan. 15, 1930	6.5 × 5 in.		
" 18, "		0.97 in.	0.7 in.
Feb. 1, "		3.4 × 1.1 in.	1.2 × 1.2 in.
" 13, "	9.7 × 5.6 in.		
" 17, "		4.7 × 1.6 in.	1.9 × 1.6 in.
Mar. 15, "	18 in.	10.3 × 2.1 in.	7.3 × 2.8 in.
" 17, "		14.8 × 2.2 in.	3.5 × 3.2 in.
Apr. 12, "	24 in.	19 × 2.5 in.	5.8 × 6.1 in.
May 13, "	36.3 in.	23.8 × 2.8 in.	
June 28, "	44.8 in.	22.1 in.	17 in.
Aug. 25, "	44.4 in.	31.6 in.	20.8 in.

Average lengths and breadths of the new thalli in the three species of *Laminaria*.

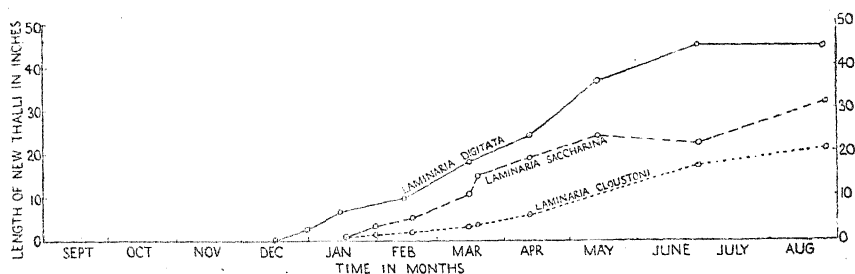
It has been suggested that sea-water is a medium containing everything necessary for the life of marine organisms with the exception of phosphate and nitrate, which are present only in minute quantities and sometimes limit plant growth. The greatest amounts of these salts occur during the winter months; they are almost completely used up during the summer, but towards the end of October they attain their maximum in the surface waters, being replenished from decaying plant and animal bodies which sink to the bottom layers. The seasonal variation of the nitrate and phosphate content of onshore waters has not yet been accurately estimated, but presumably it follows that of the open sea, though with greater fluctuation due to contamination.

The importance of temperature is illustrated by the fact that in warm bays with a southern aspect reproduction commences at an earlier date

than in the more exposed regions. In 1928, zoosporangial areas were observed on thalli at St. David's a month before they were recorded for Aberystwyth.



TEXT-FIG. 1 A. Reproduction in *L. digitata*, *L. saccharina*, and *L. Cloustoni* (Sept. 1929-Aug. 1930).



TEXT-FIG. 1 B. New growth in *L. digitata*, *L. saccharina*, and *L. Cloustoni* (1929-30).

Preliminary experiments carried on under the late Professor Robinson indicated that with increasing temperature the rate of apparent carbon assimilation decreases while that of respiration increases. It may therefore be assumed that at a certain temperature a compensating point is reached beyond which respiration exceeds assimilation. At this time of the year, also, the nitrogen content of the sea-water is at a minimum, therefore growth practically ceases. Later the nitrogen content increases while the light intensity is falling and the temperature is still high. The C/N ratio is low and sporangial formation takes place. The balance is readjusted in the zoospore, which, after a period of activity comes to rest and germinates. When the C/N ratio is again favourable, the gametophytes produce eggs and sperms.

Fertilization gives a fresh impetus to growth and small plantlets of *Laminaria* appear at the beginning of the year. Vegetative growth is also renewed in the plants of previous years and growth is rapid during the period of high nitrate and phosphate content, low temperature, and increasing light intensity.

In the present investigation an attempt was made to obtain information regarding some of the physiological relationships of *Laminaria* by observing the behaviour of the gametophytes and early stages of the sporophytes under various conditions, the microscopic size and the short duration of the gametophytes rendering it possible to grow them successfully in culture.

As yet little cultural work on the marine algae has been attempted along physiological lines, the literature consisting mainly of the description of methods and the nutrient solutions employed.

Oltmanns (15) discussed the culture and life conditions of marine algae and emphasized the importance of direct observation of the plants *in situ* as a necessary preliminary to their culture in the laboratory. He considered that the salt content of the medium, illumination, and temperature were among the chief factors influencing both propagation and distribution, and that in order to obtain normal growth, cultural conditions should imitate those of the natural habitat as closely as possible.

Noll (14) realized that phosphate and nitrogen compounds should be added to sea-water as they are essential for the formation of protoplasm and nuclear substances.

Arber (1) discussed the action of nitrates on the carbon assimilation of the marine algae. He found that additions of phosphate and nitrate to sea-water cause an inhibition of carbon assimilation. He and Duggar (6) both arranged the basic radicals ordinarily regarded as a nutrient for an autotrophic plant in the following order of toxicity:  $\text{NH}_4$ , K, Na (Arber), Ca (Duggar), and Mg.

Drew (5) found that in the case of *Laminaria*, additions of nutrient solutions containing  $\text{NaNO}_3$ ,  $\text{KNO}_3$ ,  $\text{NH}_4$ ,  $\text{NO}_3$ , and  $\text{NaHPO}_3$  to sea-water were satisfactory in promoting the growth of the gametophytes and the young sporophytes, and Killian later used a similar nutrient solution in the culture of various species of *Laminaria*. Drew's methods were as follows: Cultures were made in glass jars of 500 c.c. capacity, which were thoroughly washed and filled with sea-water to which the nutrient solution had been added. The cultures were then inoculated with small pieces of mature reproductive areas which had been gently brushed and washed in sterilized sea-water. Difficulties being experienced owing to contamination by yeast and bacteria, sub-cultures were made by pipetting off some of the flagellated gametes (zoospores). Experiments conducted in Petri dishes were not successful.

Williams (24-26) and Sauvageau (19) have grown plantlets of *Laminaria* in culture using similar methods.

Kylin (10) made cultures of *Laminaria digitata* in small glass dishes containing from thirty to forty centimetres of sea-water. Portions of thallus bearing mature sporangia were left in the water for a certain

number of days in order that the zoospores should be liberated and were then removed.

Where the initial quantity of water was changed twice per week, sporophytes were obtained in a few weeks. Where the initial quantity was not renewed and the portion of thallus was removed after two days, no fertile male or female gametophytes were formed. Occasional sporophytes were observed in cultures where the portion of thallus was allowed to remain for one or two weeks, and numerous sporophytes were formed where the thallus was removed after two days and the cultures were supplied with 0.2 per cent. sodium nitrate.

Not only did additions of nitrate favour the production of sporophytes, but they also had a marked effect on the production of pigment, gametophytes without an adequate nitrate supply having pale yellow-brown chromatophores and those with nitrate having deep brown chromatophores.

Printz (17) obtained successful cultures of *Alaria* in Petri dishes containing from 30 to 250 c.c. sea-water. He found that development was rapid in cultures containing sea-water alone and also in sea-water with additions of sodium nitrate, recommended by Kylin, but that ammonium phosphate was absolutely valueless as a source of nitrogen and had an inhibitory effect on development.

Myers (13), working on the life-histories of members of the Laminariaceae used Kylin's culture solution, that is, filtered sea-water to which had been added traces of calcium phosphate and sodium nitrate. Pieces of well-washed sori containing mature sporangia were placed in dishes of the culture solution and left there overnight in order that liberation might take place. The zoospores were allowed to settle on glass slides so that they might be more easily removed and observed.

Schreiber (20) made a detailed investigation of the effect of light intensity, temperature, and various phosphate and nitrogen compounds on some of the marine phytoplankton. From counts of *Biddulphia* and *Carteria* in culture he found that there is an optimum light intensity and an optimum temperature for the culture of these organisms. His work is also of importance in that he proved by cultural methods that the increase in the number of phytoplankton organisms present in sea-water is directly proportional to the nitrate and phosphate content of the sea-water. This confirms the assertions of Atkins (2, 3, 4) and Harvey (7, 8) that the seasonal variation in the phytoplankton population corresponds to the periodicity in the nitrate and phosphate of the sea-water. According to Schreiber, nitrogen is most effective in promoting growth and development of phytoplankton when supplied as an ammonium salt, but Harvey states that the balance of evidence suggests that almost all the nitrogen built up by marine plants into proteins is absorbed in the form of nitrates, the amount derived from other sources being small in comparison.

Ueda (22) subjected cultures of *Laminaria religiosa* to temperatures ranging between  $1.9^{\circ}\text{C}$ . and  $24.8^{\circ}\text{C}$ . He found that sporophytes were formed only between  $6.5^{\circ}\text{C}$ . and  $11.3^{\circ}\text{C}$ . and that the optimum temperature was about  $9.6^{\circ}\text{C}$ . In order to keep his temperatures constant, he used the apparatus devised by Seno and Tauti (21).

The following experimental methods have been devised in an attempt to elucidate some of the problems concerning the nutritional factors affecting development in *Laminaria*.

#### MATERIALS AND METHODS.

Material for investigation was obtained from the Constitution Hill rocks and the Pier rocks at Aberystwyth. The cultures were carried out in Petri dishes containing 25 c.c. sea-water previously filtered through a porcelain filter. Inoculation was effected in the following manner: A mature fruiting portion of thallus, having been well washed and kept overnight in a moist chamber, was placed for a few minutes in a dish of filtered sea-water where the zoospores were soon liberated. From the suspension thus obtained some of the zoospores were pipetted into large glass Petri dishes and allowed to settle on glass covers according to the method previously used by Dr. Lloyd Williams at Aberystwyth. When the zoospores had come to rest, the covers were transferred to the experimental Petri dishes. By this method the number of zoospores in each Petri dish was approximately equal.

In the preparation of nutrient solutions chemically pure salts were employed and glass apparatus was used in the distillation of water. The standard solutions used were percentage and molecular solutions, the former being employed whenever the effect of individual salts was not being estimated. Nutrient material was supplied either in one dose or at intervals of ten days by means of a 1 c.c. pipette graduated into hundredths. The initial 25 c.c. of sea-water was not renewed, so that the total amount of nutrient material added could be recorded.

The hydrogen-ion concentration of the cultural medium was not materially affected by the addition of the nutrient solutions because of the 'buffer' action of sea-water.

It was not possible to maintain a constant temperature and, in some cases, a constant light intensity throughout the experiments, the cultures being subjected to the ordinary fluctuations in the laboratory. As, however, the control cultures in all cases experienced conditions exactly similar to the remainder of the series, results obtained from the various experiments may be compared.

A brief description of the processes of germination, sexual reproduction, and growth of *Laminaria* is a necessary preliminary to an explanation

of how data were obtained for the plotting of graphs. Williams, Sauvageau, Kylin, and others have investigated and described these processes accurately, and the following record of personal observations entirely confirms their discoveries.

In culture the zoospores of *Laminaria* come to rest after a short period of activity. In less than twenty-four hours a germination-tube grows out from the spore and in one or more days the contents of the spore and germ-tube pass into the enlarged end of the latter, which becomes separated by a wall. This end region may for convenience be termed the 'effective plant'. For a length of time varying from a few days to several weeks, the plant remains in what may be called a temporary resting stage, the increase in size being very gradual and the plant maintaining a more or less spherical outline (Pl. XXXVII, Fig. 1). Growth then increases and is manifested by elongation of the effective plant and the differentiation of the male and female gametophytes.

The female gametophytes may either function as oogonia without further division or they may become elongated and septated, in which case any cell may produce an oogonium (Pl. XXXVII, Figs. 2 and 3).

The male gametophytes are smaller and are generally multicellular and branched, the branches being short and compact or thinner and more elongated (Pl. XXXVII, Figs. 2 and 3). Their growth at first appears to be more rapid than that of the female gametophytes and the chromatophores are paler, so that the sex of the gametophytes can easily be distinguished by the difference in pigmentation as well as by the smaller diameter of the male. Any cell can function as an antheridium, the end cells being first converted. As the process of antheridial formation proceeds and the sperms are discharged, the male gametophytes become gradually invisible.

According to Williams the single egg produced by an oogonium is fertilized by a spermatozoid after emergence from the oogonium. The oospore either remains attached to the empty oogonium or becomes free. It elongates rapidly, forming a thin septated filament which later becomes a flattened expansion tapering at apex and base (Pl. XXXVII, Fig. 4).

The growth of the gametophytes before the differentiation of the sexes was recorded by camera-lucida drawings of sample populations and by linear measurements of these drawings. Subsequent measurement of the male plants was impossible owing to branching and irregularity of growth, but in most cases the growth of the female was recorded until the unfertilized oogonia were liberated. Data were similarly obtained of the early growth of the sporophytes.

Linear measurements were in all cases taken as measurements of growth, since it was observed that in all cultures except those in Section III increase in length was accompanied by increase in volume.

In early experiments twenty-five plants from each culture were drawn,

but later the number was increased to fifty. The arithmetical averages of the measurements are recorded in terms of  $\mu$ .

Records of the fertility of the gametophytes were made by noting the dates of the first appearance of sexual organs and the percentages of mature fertile plants.

The data have in several cases been recorded graphically, the percentages of the mature fertile plants and also the lengths of the gametophytes and sporophytes being plotted against time.

It was found convenient to divide the work into sections dealing respectively with the most favourable culture medium, the chief determining factor for growth and sexual reproduction, and the effect of varying light conditions.

Since early experiments indicated that the gametophytic growth of the three species of *Laminaria* differs to some extent, *Laminaria saccharina* was used exclusively in the later sections of the work.

#### SECTION I.

##### *To Determine the Concentration of a Combined Nutrient Solution most Favourable for the Growth and Reproduction of the Gametophytes of Laminaria.*

Preliminary work showed that additions of potassium nitrate and potassium phosphate accelerated the growth and the development of *Laminaria* gametophytes in culture, and the abundance of iodine present in the larger members of the Phaeophyceae suggested that it might be a factor of some importance. It was, therefore, decided that these substances should be included in a nutrient solution, and in order to ascertain the most favourable concentrations, two solutions were prepared containing respectively 0.1 per cent. and 0.01 per cent. of each of the salts of potassium nitrate, potassium dihydrogen phosphate, and potassium iodide. The solutions were supplied to the initial 25 c.c. of sea-water in quantities varying between very low and fairly high limits.

Two series of experiments were set up on November 21st and 28th, 1928, using *L. digitata* and *L. saccharina* respectively.

The first experiment consisted of two parallel series of cultures of *L. digitata*, set up according to the method previously described. The combined nutrient solution was supplied to the first series, A, but once, and to the second series, B, at intervals of ten days. The size of the additions ranged from 0.1 c.c. of a 0.01 per cent. solution to 0.5 c.c. of a 0.1 per cent. solution, the proportions of K, N, P, and I being as indicated in Table II A.

The upper limit of nutrient substances which allows of the germination of the zoospore was not reached, for in all cases the appearance of germ-



TABLE II A.

Cultures.		c.c. combined nutrient solution added to cultures.	Amount of the various elements contained in one addition of the nutrient solution.			
Series A.	Series B.		mg. K.	mg. N.	mg. P.	mg. I.
<i>a</i>	<i>a'</i>	0.1 of a 0.01 % solution	0.0091	0.0014	0.0023	0.0076
<i>b</i>	<i>b'</i>	0.2     "     "	0.0182	0.0028	0.0046	0.0142
<i>c</i>	<i>c'</i>	0.5     "     "	0.045	0.007	0.0115	0.038
<i>d</i>	<i>d'</i>	0.1     0.1 %     "	0.091	0.014	0.023	0.076
<i>e</i>	<i>e'</i>	0.15     "     "	0.137	0.021	0.036	0.114
<i>f</i>	<i>f'</i>	0.2     "     "	0.182	0.028	0.046	0.142
<i>g</i>	<i>g'</i>	0.3     "     "	0.273	0.049	0.069	0.228
<i>h</i>	<i>h'</i>	0.4     "     "	0.364	0.056	0.092	0.304
<i>i</i>	<i>i'</i>	0.5     "     "	0.45	0.07	0.115	0.38

Amount of nutrient material supplied in Expt. I to the cultures of *Laminaria digitata*, in the form of a combined nutrient solution.

Series A received one addition ; series B received additions at intervals of 10 days.

TABLE II B.

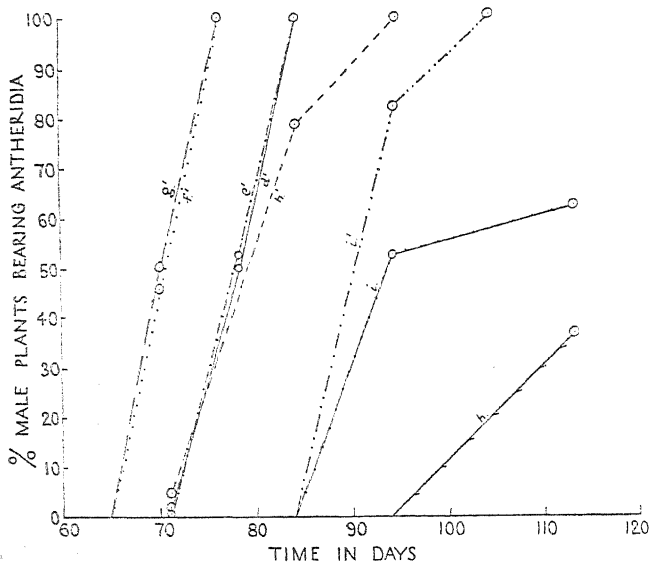
Percentages of mature, fertile male and female gametophytes of *L. digitata*. (Expt. I.)

Days after the commencement of the experiment.		65.	71.	76.	78.	80.	84.	94.	100.	104.	110.	113.
Cultures.												
A.	<i>a</i>											
	<i>b</i>											
	<i>c</i>											
	<i>d</i>											
	<i>e</i>											
	<i>f</i>											
	<i>g</i>											
	<i>h</i>											36.1
	<i>i</i>											61.9
B.	<i>a'</i>											
	<i>b'</i>											
	<i>c'</i>											
	<i>d'</i>									1.1	5.5	
	<i>e'</i>			2	50	100				32	34.5	
	<i>f'</i>	Few	45.9	100	25	52.3	100	13.3	73.3	87.6	93.4	
	<i>g'</i>	Few	50	100		48.2	56.3	87	100			
	<i>h'</i>					6.4	12.2	76.2	100			
	<i>i'</i>		5				78.9	100		100		

The percentages of fertile female gametophytes are given in italics.

tubes was recorded. There was, however, a tendency for the terminal enlargement of the germ-tube to be retarded in all cultures at the upper and lower limits of the series. In all cultures the plants remained in the

temporary resting phase for a few weeks, during which period there was very little growth. When, as a result of a uniform increase in size a diameter of about  $8\mu$  was reached, elongation recommenced, the date on which it took place varying in the different cultures.



TEXT-FIG. 2 A. Production of antheridia of *L. digitata*. (Section I, Expt. I.)

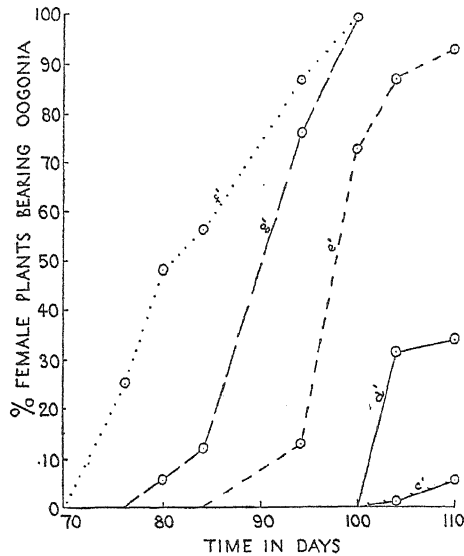
In Series A, in fifty-one days, growth was greatest in the culture at the upper end of the series, receiving 0.5 c.c. of the nutrient solution, whereas in Series B it occurred in cultures which had received three additions of 0.25 c.c. and 0.3 c.c. respectively. With increasing and decreasing quantities growth was correspondingly less.

After fifty-six days the cultures were removed to a bench where they received light of greater intensity, and the growth and development became immediately accelerated, antheridia being observed in the most advanced cultures, *g'* and *f'*, in fifteen days' time.

From Table II B and Text-figs. 2, A, B, it may be seen that the fertility of the male and female gametophytes was greatest in those cultures in which growth was most rapid, *g'* and *f'*. Antheridia were produced in *h'* and *i'* which received additions of 0.4 c.c. and 0.5 c.c. of the nutrient solution per ten days at an earlier date than in the cultures supplied with less than 0.2 c.c., but while the cultures *h'* and *i'* produced no sporophytes, a high percentage was recorded for *e'* receiving 0.15 c.c. and low percentages for *d'* and *c'* receiving 0.1 c.c. and 0.05 c.c. per ten days.

In Series A, in seventy-one days, a few antheridia were present in the cultures receiving the largest additions of nutrient, *h* and *i*, but no sporophytes were formed.

Unfortunately, before the experiment was concluded the cultures were killed by exposure to direct sunlight. The data obtained are, how-



TEXT-FIG. 2 B. Production of oogonia of *L. digitata*. (Section I, Expt. I.)

ever, sufficient to justify certain conclusions which will be discussed in conjunction with Experiment II.

TABLE III A.

Cultures.		c.c. combined nutrient solution added to cultures.	Amount of the various elements contained in one addition of the nutrient solution.			
Series A.	Series B.		mg. K.	mg. N.	mg. P.	mg. I.
<i>a</i>	<i>a'</i>	0.1 of a 0.01 % solution	0.0091	0.0014	0.0023	0.0076
<i>b</i>	<i>b'</i>	0.1 of a 0.1 % "	0.091	0.014	0.023	0.076
<i>c</i>	<i>c'</i>	0.25 " "	0.23	0.035	0.06	0.19
<i>d</i>	<i>d'</i>	0.5 " "	0.45	0.07	0.12	0.38
<i>e</i>	<i>e'</i>	0.75 " "	0.68	0.105	0.18	0.57
<i>f</i>	<i>f'</i>	1.0 " "	0.91	0.14	0.23	0.76

Amount of nutrient material supplied to the cultures of *L. saccharina* (Expt. II) in the form of a combined nutrient solution.

Series A received one addition; Series B, additions at intervals of 10 days.

In the second experiment, cultures of *L. saccharina* received the combined nutrient solution over a range of 0.1 c.c. of a 0.01 per cent. solution to 1.0 c.c. of a 0.1 per cent. solution, supplied as before, either in one addition or at intervals of ten days (Table III A).

The larger additions of nutrient exercised a slightly inhibitory effect

on germination, but, in three days, in all cultures the contents of spore and germ-tube had passed into the enlarged end of the latter.

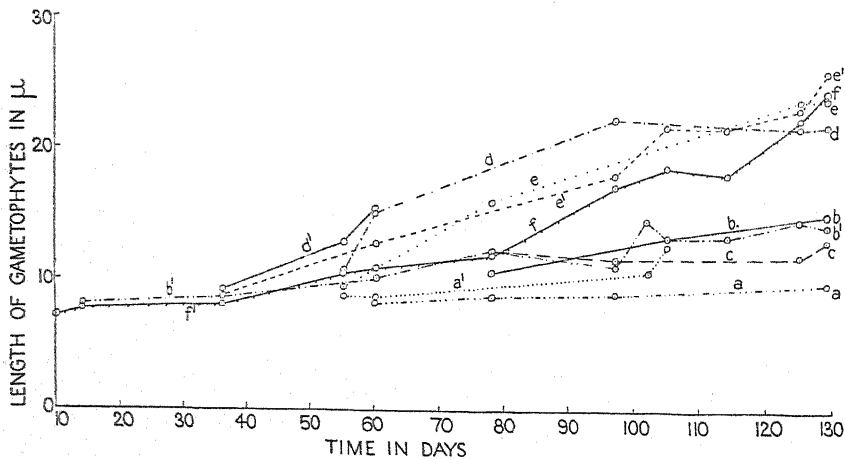
The temporary resting period was prolonged also in this series owing to the low light intensity experienced during the first fifty-six days. Growth was observed as is recorded in Table III B and Text-figs. 3, A, B.

TABLE III B.

Length of gametophytes and sporophytes of *L. saccharina* in  $\mu$ .

Days after the com- mencement of the Expt.	10.	14.	36.	55.	60.	78.	97.	102.	105.	114.	125.	129.	135.
Cultures.													
<i>a</i>					8.2	8.7	8.8					9.6	
<i>b</i>					10.5	11.5						14.7	
<i>c</i>					12.1	11.3					11.7	12.8	
<i>d</i>			10.5	15		22					21.6	21.5	
<i>e</i>			9.3		15.8	38.6		53.3			23.4	71	23.5
<i>f</i>												90	19.7
<i>a'</i>				8.7	8.7			10.5	12.5				
<i>b'</i>		8	8.6		10	12.2	11.0	14.4	13.1	13.1	14.4	14	
<i>c'</i>					11.6		93.9			164.4	222.2		290.5
<i>d'</i>			9.2	12.8	15.3		94.2			151.1	205.8		200
<i>e'</i>				8.7	12.6		17.9	17.2	21.5	21.4	22.8	25.6	
<i>f'</i>	7.2	7.7	8	10.4	10.8	11.8	17	18.4	17.9	22.0	22.0	24.2	

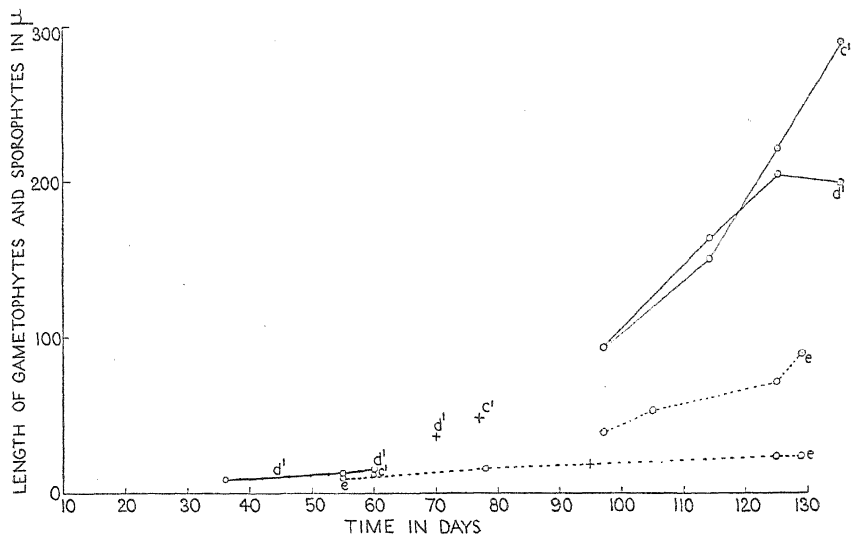
The lengths of the sporophytes are stated in italics.



TEXT-FIG. 3 A. Growth of gametophytes of *L. saccharina*. (Section I, Expt. II.)

It must be remembered that light intensity and temperature were increasing during the period of the experiment, but the conditions with regard to these factors were strictly comparable for all cultures.

Antheridia were first observed in fifty-nine days and sporophytes in sixty-seven days after the commencement of the experiment. The data regarding the production of mature sexual organs are given in Table III C



TEXT-FIG. 3 B. Relative growth-rates of gametophytes and sporophytes of *L. saccharina*. (Section I, Expt. II.)

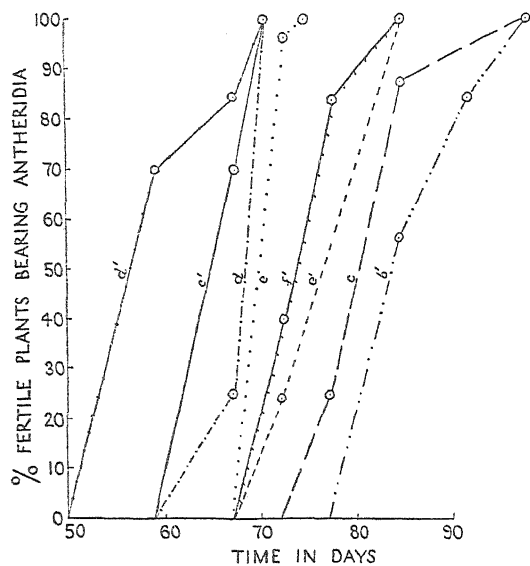
and Text-figs. 3, C, D. Pl. XXXVII, Figs. 5-7, Pl. XXXVIII, Figs. 9-11 show the difference in growth and development between members of Series B ninety-four days and a hundred and sixteen days after the liberation of the zoospores.

TABLE III C.

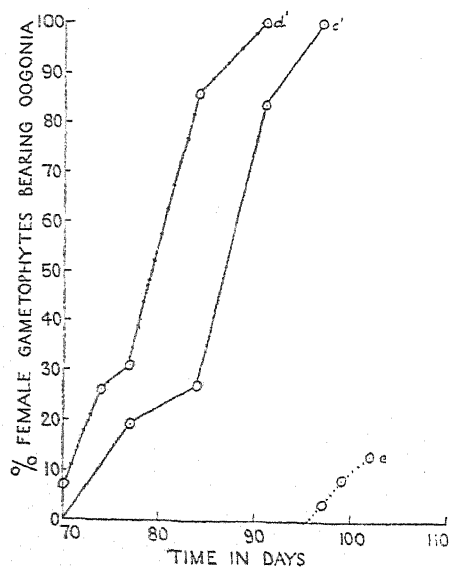
Percentages of mature, fertile male and female gametophytes in the cultures of *L. saccharina*.

Days after the commencement of the Expt.	59.	67.	70.	72.	74.	77.	84.	91.	97.	99.	102.
Cultures.											
a											
b											
c						25	87.6		100		
d		25	100								
e				96.1	100				3.8	8.7	13.2
f											
a'											
b'							56.1	84.1	100		
c'		70	100			19.7	27	83.8	100		
d'	70	84.5	100	7.2	26.3	31.1	86	100			
e'				24			100				
f'				40		84	100				

The percentages of fertile female gametophytes are given in italics.



TEXT-FIG. 3 C. Production of antheridia of *L. saccharina*. (Section I, Expt. II.)



TEXT-FIG. 3 D. Production of oogonia of *L. saccharina*. (Section I, Expt. II.)

*Discussion of results.*

Examination of the data obtained from these two experiments leads to the following conclusions:

The fact that zoospores, provided with sea-water alone or with sea-water plus minute quantities of nutrient material, put out germ-tubes, pass into the temporary resting stage and remain in that condition, increase in size only occurring where nutrient is added in sufficient quantity, suggests that one or more of the salts present in the nutrient solution are essential for further development.

It is noteworthy that in the development of the gametophyte there is a temporary resting stage during which the effective plant increases slowly in size. The fact that (*a*) a certain diameter is reached before elongation commences and that (*b*) this period is shorter where nutrient is supplied in fairly large quantities, indicates the possibility that an accumulation of nutrient material within the plant is necessary for further development.

It is known that in sea-water, phosphate, nitrate, and iodide are present only in very minute quantities, but diffusion must be greatly facilitated by the perpetual motion of the sea-water.

A minimum amount of growth was found in cultures with least nutrient, and increase in nutrient was accompanied by increase in growth. The latter attained a maximum in cultures to which nutrient was added in relatively large quantities at definite intervals.

It is clear that growth depends both on the size of the initial addition of nutrient and also on subsequent additions, for, eighty days after the commencement of Experiment II, the culture supplied with one addition of 0.75 c.c. was in advance of that supplied with eight additions of 0.1 c.c., but the cultures which received 0.25 c.c. and 0.5 c.c. on each of eight days were even more advanced.

There is evidence that a single large addition of nutrient exercises an inhibitory effect on germination and growth. Where the amount is not excessive the effect gradually diminishes, but, where the addition is repeated, there is a marked decrease in the rate of growth and also in the capacity for complete development. The branching of the male gametophytes in Experiment II varied in the different cultures, being greatest in those in which growth was most rapid (*e*, *d*, *d'*, *c'*). In the cultures which developed less rapidly (*b'*) and in those at the upper end of the series (*f*, *e'*, *f'*) there was practically no branching.

It may be seen from Tables III, A, B, and C, that the amount of nutrient substances most favourable for growth was also most favourable for the production of antheridia and oogonia. The maximum amount of nutrient substances which allow of the production of reproductive organs was not reached, but the sporophytes of *L. saccharina* formed at the upper limit of

Series B were of rare occurrence and were situated under the covers or in the surface film. According to Livingston, growth in the surface film is not strictly comparable to that under conditions of complete submergence, since in the former case the organisms are not wholly exposed to the effects of their media. Hence the growth of the sporophytes in the situations mentioned above may be due to the fact that the germlings were only partially in contact with the highly concentrated medium, which consequently exercised a less marked inhibition on development.

It is evident from Text-figs. 2, A, B, and Text-figs. 3, C, D, that antheridia were formed over a much wider range of nutrient concentration than were the oogonia. In *L. saccharina* all male plants in *b'* and *f'* were fertile, while few oogonia were produced. Growth of the fertile plants in *b'* ceased before oogonial formation and did not recommence for some time, but in *e'* and *f'*, after the shedding of sperms, the female gametophytes increased in size and became septated (Pl. XXXVIII, Fig. 11).

The cultures supplied with one fairly large addition of nutrient solution developed rapidly at first, but later their activity decreased and the full quota of the sporophytes was not formed, probably owing to the fact that the salts were completely used up. There was practically no further growth of any kind in these cultures.

No definite conclusions have been made concerning the nutritional relationships of the sporophytes of *Laminaria* since they were not produced over a sufficiently wide range of nutrient.

Since in this section such marked correlations were obtained between the growth and the development of the *Laminaria* gametophytes and the nutrient content of the medium, it was thought necessary to determine the effect of the individual salts present in the combined nutrient solution. Therefore, in Section II, molecular solutions of the various salts were added to the cultures in different quantities.

## SECTION II.

### *The Individual Effect of Potassium Phosphate, Potassium Nitrate, and Potassium Iodide on Growth and Reproduction of the Gametophytes and Early Growth of the Sporophytes of Laminaria.*

Data obtained from preliminary experiments in which the three salts were respectively omitted from the culture medium suggested that nitrate and phosphate are essential for growth and reproduction while iodide is non-essential. To substantiate these results and to find the amount of each salt necessary for growth, three series of cultures were set up. Molecular solutions of the various salts were employed as sources of nutrient material and additions were repeated at intervals of ten days. In each



TABLE IV A.

Cultures.	KNO <sub>3</sub> .	c.c. of the nutrient solutions added to the cultures.	KH <sub>2</sub> PO <sub>4</sub> .	KI.	Amounts of the various elements contained in one addition of the nutrient solutions.			
					mg. K.	mg. N.	mg. P.	mg. I.
<i>a</i>	0.5 of a 0.01 M. solution		—	0.3 of a 0.01 M. solution	0.32	0.07	—	0.38
<i>b</i>	"	0.1 of a 0.0001 M. solution	"	"	0.3204	"	0.00031	"
<i>c</i>	"	0.1 of a 0.001 M. solution	"	"	0.3239	"	0.0031	"
<i>d</i>	"	0.1 of a 0.01 M. solution	"	"	0.359	"	0.031	"
<i>e</i>	"	0.25 "	"	"	0.42	"	0.078	"
<i>f</i>	"	0.5 "	"	"	0.51	"	0.155	"
<i>g</i>	"	0.75 "	"	"	0.62	"	0.233	"
<i>h</i>	"	1.0 "	"	"	0.72	"	0.31	"
<i>i</i>	"	1.5 "	"	"	0.92	"	0.465	"

Nutrient materials supplied to the cultures of the phosphate series at intervals of 10 days.

series the amount of two salts was kept constant, whilst the third was varied.

*Phosphate series.*

Duplicate series of cultures of *L. saccharina* were set up on March 14, 1929. Equal quantities of  $\text{KNO}_3$  and KI were supplied to all cultures, while the additions of  $\text{KH}_2\text{PO}_4$  were varied between 0 c.c. and 1.5 c.c. of a 0.01 molecular solution (Table IV A).

The results obtained from this experiment were very striking. Growth during the first fourteen days was fairly uniform in all cultures, but on the eighteenth day it was observed that increase in the concentration of phosphate had produced a corresponding increase in the length of the gametophytes (Text-fig. 4, Table IV B).

TABLE IV B.

Days after the commencement of the Expt.	Length of gametophytes in $\mu$ .				
	3.	10.	21.	29.	40.
Cultures.					
<i>a</i>	7.5	10.6	11.3	12.6	13.9
<i>b</i> <i>b'</i>	—	—	10.9	13.3	14.1
<i>c</i> <i>c'</i>	—	—	11.4	13.1	19
<i>d</i> <i>d'</i>	—	—	11.1	13.6	15.9
<i>e</i> <i>e'</i>	—	—	11.4	14.4	16.8
<i>f</i> <i>f'</i>	—	—	12.7	16.5	37.4
<i>g</i> <i>g'</i>	—	—	11.4	17.4	51
<i>h</i> <i>h'</i>	—	—	14.5	29.2	44.3
<i>i</i> <i>i'</i>	—	—	16.9	41	60.5

Variation in the length of the gametophytes of *L. saccharina* supplied with different amounts of phosphate.

The following peculiarities were also observed :

I. Light appeared to have a directive influence on the growth of the female gametophytes.

II. The growth differed from that observed in previous experiments. Instead of assuming the egg-like form prior to the formation of oogonia, the plants put out germ-tubes, which, in the cultures at the upper end of the series, attained lengths many times the diameters of the original cell.

Pl. XXXVIII, Figs. 12-14, illustrate this point and also bring out the difference in the amount of growth in the respective members of the series.

III. There was very little difference in the pigmentation of male and female gametophytes, whereas normally the female is much more deeply pigmented than the male.

The peculiar method of growth cannot be ascribed to excess phosphate as the same phenomenon occurred elsewhere in cultures receiving the combined nutrient solution. This will be discussed in Section III in connexion with light intensity and temperature.

It is clear, however, that phosphate bears a distinct relation to growth.

Antheridia were formed simultaneously in all cultures except those supplied with little or no phosphate. There were very few cases of fertilization in the cultures. Sporophytes occurred in several members of the series and apparently bore no relation to the phosphate content of their respective media.

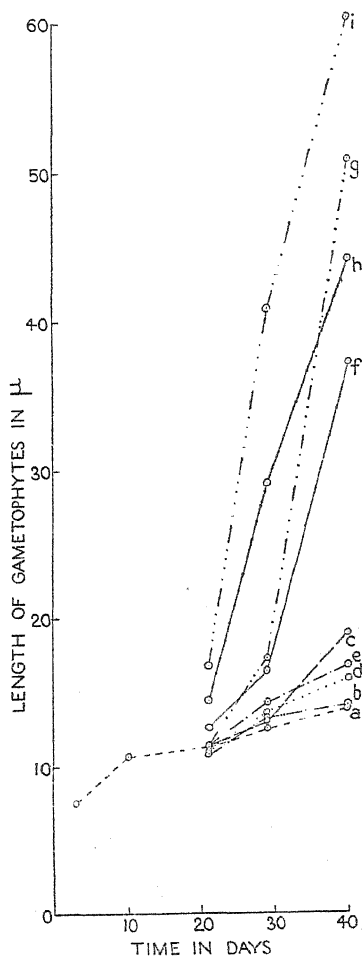
The results obtained are markedly different from those of Section I, growth in the phosphate series being most rapid when additions of nutrient were greater than any supplied in the previous experiments. From Tables III A and IV A it can be seen that neither potassium nor phosphorus were responsible for the retarded growth and the lack of fertilization which occurred in *L. saccharina*, *e'* and *f'*, Section I, Experiment II. These facts can therefore only be explained with reference to the iodide and nitrate series which will be considered next.

#### Iodide series.

Cultures of *L. Cloustoni* were set up on March 14, 1929. All were supplied with equal additions of  $\text{KNO}_3$  and  $\text{KH}_2\text{PO}_4$ , the quantity of KI being varied between 0.0 c.c. and 1.0 c.c. of a 0.01 molecular solution (Table V).

Little difference between the cultures was visible during the early stages of development, but later it was observed that growth was slightly retarded in the cultures supplied with phosphate and nitrate alone. Antheridia and oogonia were, however, formed in this culture, and eventually sporophytes were produced, though at a later date than in the other members of the series.

It was impossible to differentiate between the cultures which had received additions of iodide with the exception of *b* and *c*, Table V, to which were added the minimum quantities and in which growth was rather more rapid. Also, in the culture supplied with the maximum addition of iodide, growth was retarded and ultimately ceased.



TEXT-FIG. 4. Growth of gametophytes of *L. saccharina*. (Phosphate series, Section II.)

TABLE V.

Cultures.	c.c. nutrient solutions added to cultures.			Amounts of the various elements contained in one addition of the nutrient solutions.				
	KNO <sub>3</sub> .	KH <sub>2</sub> PO <sub>4</sub> .	KI.	mg. K.	mg. N.	mg. P.	mg. I.	
a	0.3 of a 0.01 M. solution	0.3 of a 0.01 M. solution	—	0.235	0.042	0.093	—	
b b'	"	"	0.1 of a 0.001 M. solution	0.235	"	"	0.00127	
c c'	"	"	0.1 of a 0.001 M. solution	0.239	"	"	0.0127	
d d'	"	"	0.1 of a 0.01 M. solution	0.271	"	"	0.127	
e e'	"	"	0.25 "	0.333	"	"	0.323	
f f'	"	"	0.5 "	0.431	"	"	0.635	
g g'	"	"	1.0 "	0.626	"	"	1.269	
g g'	"	"	"	"	"	"	"	

Nutrient materials added to the cultures of the iodide series at intervals of 10 days.

Nutrient materials added to the cultures of the iodide series at intervals of 10 days.

TABLE VI.

Cultures.	c.c. nutrient solutions added to cultures.			Amounts of the various elements contained in one addition of the nutrient solutions.			
	KNO <sub>3</sub> .	KH <sub>2</sub> PO <sub>4</sub> .	KI	mg. K.	mg. N.	mg. P.	mg. I.
<i>a</i>	—	0.3 of a 0.01 M. solution	0.1 of a 0.01 M. solution	0.156	—	0.093	0.1269
<i>b</i>	0.1 of a 0.01 M. solution	"	"	0.195	0.014	"	"
<i>c</i>	0.25 "	"	"	0.254	0.035	"	"
<i>d</i>	0.5 "	"	"	0.352	0.07	"	"
<i>e</i>	0.75 "	"	"	0.450	0.105	"	"
<i>f</i>	1.0 "	"	"	0.547	0.14	"	"
<i>g</i>	1.5 "	"	"	0.743	0.21	"	"
<i>g'</i>	"	"	"	"	"	"	"

Nutrient materials supplied to the cultures in the nitrate series at intervals of 10 days.

The above results suggest that a trace of iodide is essential for optimum development; that increase in concentration does not produce a corresponding effect on growth and reproduction, and that high concentrations are toxic.

*Nitrate series.*

On March 16 a third series of cultures was set up to which were added equal amounts of iodide and phosphate and from 0 c.c. to 1.5 c.c. of a 0.01 M. solution of  $\text{KNO}_3$  (Table VI).

The results obtained are of interest in conjunction with those previously described. Antheridia appeared to develop simultaneously in all cultures with the exception of the controls without nitrate, but sporophytes were first observed in *d* and *e* supplied respectively with 0.5 c.c. and 0.75 c.c.  $\text{KNO}_3$  per ten days. Sporophytes occurred later in all cultures except the controls, but maximum production and growth occurred in *c* and *e'*.

Pl. XXXVIII, Figs. 15 and 16, Pl. XXXIX, Figs. 17 and 18, illustrate both these points. The photographs were taken seventy-five days after the commencement of the experiment and show that, up to a certain concentration, increase in the amount of nitrate is accompanied by increase in growth and fertility, but that further increase inhibits both these processes.

GENERAL DISCUSSION.

Analyses of the results obtained from these three experiments show that:—

I. Small additions of phosphate are necessary for growth and reproduction of *Laminaria* gametophytes in culture. Increase in the amount of the addition is accompanied by a corresponding increase in growth. Antheridia and oogonia are produced over a wide range of phosphate concentration.

II. Additions of iodide are non-essential for growth and the production of antheridia and oogonia, but small additions accelerate development. High concentrations are toxic.

III. Additions of nitrate are necessary for growth and reproduction in culture. Antheridia and oogonia are formed only within a restricted range of nitrate concentration.

From these data and from observation of Pl. XXXVII, Figs. 5 and 7, Pl. XXXVIII, Figs. 8-16, Pl. XXXIX, Figs. 17 and 18 it can easily be seen that the nitrate series bears the closest relation to the series of Section I.

It may be concluded that provided a certain amount of phosphate and a trace of iodide are present, the quantity of nitrate present in the culture medium is the chief factor that controls growth of the gametophytes and sporophytes and the reproduction of the former.

It is true that the range of nitrate within which sporophytes were produced was greater in the nitrate series than that of the series of Section I. Also the amount of nitrate most favourable for development was higher in the former series. This, however, may be due to the difference in the source of material, or the increase in intensity or duration of light and the increase in temperature, which would allow of the utilization of greater quantities of nitrate.

### SECTION III.

Preliminary series of cultures were set up in daylight and under conditions of artificial illumination in order to ascertain the effect of light and to obtain further data regarding the effect of nitrate and phosphate on the development of the *Laminaria* gametophytes.

In all experiments three or more series of cultures of *L. saccharina* were set up and these were subjected respectively to light of different intensities. One series was placed in daylight while the remainder were artificially illuminated in a dark room according to the method described by Schreiber.

Each series consisted of cultures with the same range of phosphate and nitrate content. Comparisons could, therefore, be made between:

A. Varying amounts of these salts at the same light intensity.

B. The same amounts of salts at different light intensities.

Data obtained from a rough preliminary experiment indicated that light of a certain intensity was necessary for the production of sporophytes. A second experiment was, therefore, conducted in the following manner:

Four series of cultures, A, B, C, and D were set up on January 3, 1930. Series A was placed in daylight, and the other series were placed at distances of 10.2, 16, and 21.8 inches from a 300 c.p. Osram electric lamp for ten hours per day and received light as measured by the Bee Metre in the ratio of 8:2.5:1. The cultures were illuminated from above, a glass trough filled with water being placed immediately below the lamp in order to absorb the heat rays. The temperature was kept as low as possible by the constant renewal of the water in the trough, but fluctuated between 4° and 16° C.

Each series consisted of twelve cultures including a control in sea-water. The quantities of  $\text{KH}_2\text{PO}_4$ ,  $\text{KNO}_3$ , and KI added to the cultures are given on Table VII A.

Growth was rapid in all the cultures except the controls, and antheridia were formed simultaneously in all cultures receiving artificial illumination twelve days after the liberation of the zoospores and a few days prior to their formation in the daylight series. A few growth measurements were recorded (Table VII B, Text-fig. 5 A).

Data regarding the production of sporophytes are given on Table VII C and Text-figs. 5 B and C. It will be seen that no sporophytes

TABLE VIIA.

Cultures.	c.c. of the nutrient solutions added to the cultures.				Amounts of the various elements contained in one addition of the nutrient solutions.				
	KNO <sub>3</sub> .	KH <sub>2</sub> PO <sub>4</sub> .	KI.		mg. K.	mg. N.	mg. P.	mg. I.	
Control I	—	—	—		—	—	—	—	
Control II	0.5 of a 0.01 M. solution	—	0.1 of a 0.001 M. solution		0.199	0.07	—	0.01269	
<i>a</i>	0.25 of a 0.01 M. solution	0.5 of a 0.01 M. solution	"	"	0.199	—	0.155	"	
<i>b</i>	0.5 "	"	"	"	0.2972	0.035	0.155	"	
<i>c</i>	0.75 "	"	"	"	0.3949	0.07	0.155	"	
<i>d</i>	1.0 "	"	"	"	0.4927	0.105	0.155	"	
<i>e</i>	0.75 "	"	"	"	0.5904	0.14	0.155	"	
<i>f</i>	0.5 "	"	"	"	0.3949	0.105	0.078	"	
<i>g</i>	0.5 "	1.0 "	"	"	0.2972	0.07	0.078	"	
			"	"	0.5904	0.07	0.31	"	
Nutrient materials supplied at intervals of 10 days to the cultures of Series A, B, C, and D, Section III, Expts. II and III.									

Nutrient materials supplied at intervals of 10 days to the cultures of Series A, B, C, and D, Section III, Expts. II and III.

TABLE VII B.

Length of gametophytes and sporophytes in  $\mu$  (Section III, Expt. II).

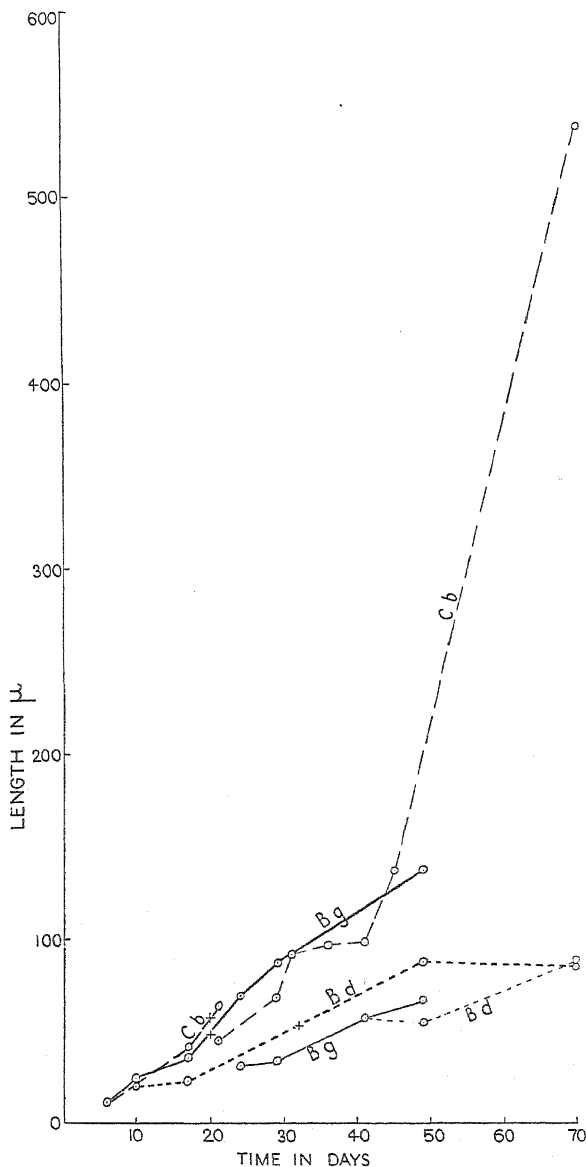
Days after the commencement of the Expt.		6.	10.	17.	21.	24.	29.	31.	36.	41.	45.	49.	70.
Cultures.													
Series B. Control.	10.9												
Series C. Control.	11.9												
Series B. <i>b</i>			20	22.3						56.4		54.88	88
Series C. <i>b</i>				44			68	90.8	96.8	98	137.2	88	85.6
Series B. <i>g</i>		11.6	24.6	35.3		31.6	86.4			56.8		66.4	540
						68.8						138.8	

The lengths of the sporophytes are stated in italics.

TABLE VIIc.  
Percentages of mature, fertile female gametophytes in Section III, Expt. II.

[illegible]

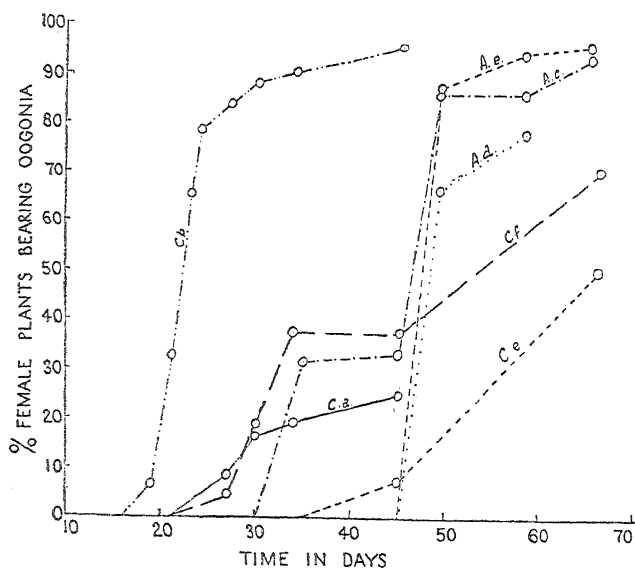




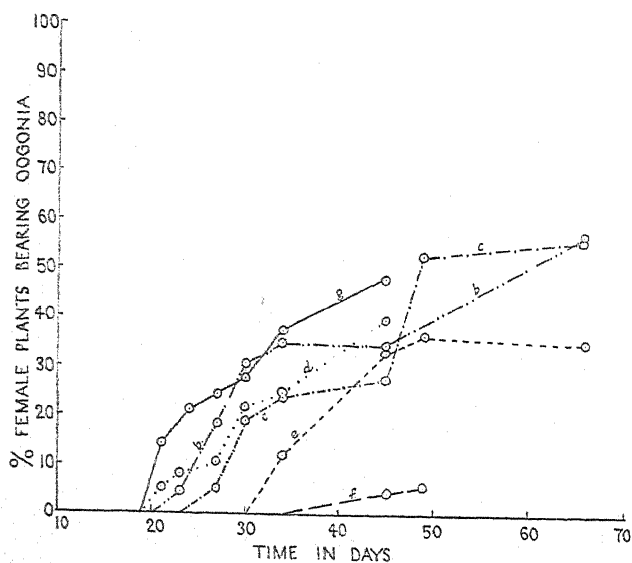
TEXT-FIG. 5 A. Growth of gametophytes and sporophytes of *L. saccharina*.  
(Section I, Expt. II.)

Thick lines represent growth of gametophytes; fine lines represent sporophytes.  
x marks the day on which oogonia were first liberated in the respective cultures.  
Capital letters indicate series; small letters indicate cultures.

occurred in the controls in any series except Series B, and few were recorded for any culture of Series D.



TEXT-FIG. 5B. Production of oogonia of *L. saccharina*. (Section III, Expt. II, Series A and C.)



TEXT-FIG. 5C. Production of oogonia of *L. saccharina*. (Section III, Expt. II, Series B.)

A duplicate series set up on January 23 gave the following results (Tables VIII A and VIII B, Text-fig. 6) :—

TABLE VIII A.

Length (in  $\mu$ ) of gametophytes and sporophytes in some cultures of Expt. III.

Days after the commencement of the Expt.		3.	20.	21.	28.	29.	36.	48.	49.	58.	59.
Series.	Culture.										
A.	<i>c</i>			11.1				80.2 35.6		118.0 77.2	
	<i>g</i>			13.6		18.2	41.2 31.6	95.2 65.6			161.6
B.	<i>d</i>	8.7	16.4		36 18.4		46.2 36	78.8 48.4			

The lengths of the sporophytes are stated in italics.

TABLE VIII B.

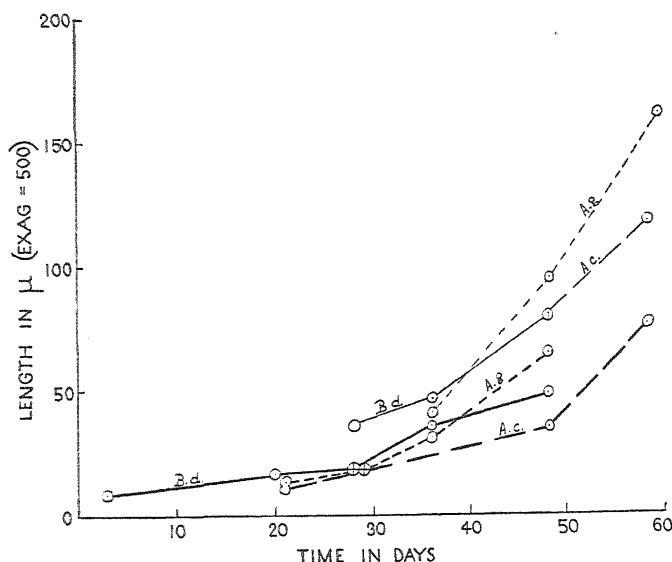
Percentages of mature, fertile female gametophytes in Series A and B, Expt. III.

Days after the commencement of the Expt.		24.	28.	34.	37.	44.	45.	51.	56.	57.
Series.	Culture.									
A.	<i>a</i>				15.3					
	<i>b</i>			4.6						
	<i>c</i>			4.1		20.8		23	27	
	<i>d</i>					7.2			14.4	
	<i>g</i>		10.8	17.3		22.7		28.9	24.1	
B.	<i>c</i>	3.3	7	25.2			37.5			51.3
	<i>d</i>		5.7	19.3			20.8			24.1
	<i>g</i>		13.3							

Antheridia were formed in all cultures except the controls, but their production was retarded in Series D, which received the lowest light intensity. In twenty days a few sporophytes were observed in all cultures in Series B except the controls without phosphate and the cultures supplied with the smallest additions of phosphate. Four days later they were formed in the daylight series and occasional sporophytes were recorded for culture *g*, Series C, and culture *b*, Series D. It was difficult to estimate the actual percentages present in the various cultures owing to the filamentous nature of the growth. The numbers did not, however, increase after the beginning of March owing to the fact that it became impossible to keep the temperature low.

In a fourth experiment set up on February 22, light of greater intensity was supplied to the cultures by means of a 500 candle-power lamp ;

also a wider range of phosphate and nitrate was tested (Table IX A). The experiment consisted of three series of cultures, Series A being placed in daylight, Series B receiving a light intensity twice as high as that to which



TEXT-FIG. 6. Growth of gametophytes and sporophytes of *L. saccharina*. (Section III, Expt. III.)

Thick lines represent growth of gametophytes; thin lines growth of sporophytes. x marks the day on which oogonia were first liberated.

Capital letters represent the series; small letters represent cultures.

Series B in the previous experiments was subjected, and Series C, which corresponded to the former Series B.

Where there was no addition of salts, development did not proceed beyond the initial stages of germination. Data were limited owing to the high temperature, but sporophytes were formed in Series A and B (Table IX B).

Data from this section are rather few, but certain conclusions are indicated.

#### A. At all light intensities.

I. There was practically no growth or development in the controls in sea-water (Pl. XXXIX, Fig. 19).

II. Supplied with nitrate but no phosphate there was little growth, the male gametophytes being but slightly branched and the female gametophytes retaining their spherical outline (Pl. XXXIX, Fig. 20). Gametophytes of both sexes were deeply pigmented. Phosphate appeared to be essential for further growth and development. The slight quantity present

TABLE IX A.

Amount of the various elements contained in one addition of the three nutrient solutions.

c.c. nutrient solutions added to the cultures.

Cultures.

	KNO <sub>3</sub> .	KH <sub>2</sub> PO <sub>4</sub> .	KI.	mg. K.	mg. N.	mg. P.	mg. I.
Control	1.0 of a 0.01 M. solution	1.0 of a 0.01 M. solution	0.1 of a 0.001 M. solution	0.7859	0.14	0.31	0.01269
<i>a</i>	0.75 "	" "	" "	0.6882	0.105	0.31	"
<i>b</i>	0.5 "	" "	" "	0.5904	0.07	0.31	"
<i>c</i>	0.75 "	" "	" "	0.5904	0.105	0.233	"
<i>d</i>	0.5 "	0.75 "	" "	0.4927	0.07	0.233	"
<i>e</i>	1.0 "	" "	" "	0.5904	0.14	0.155	"
<i>f</i>	0.75 "	" "	" "	0.4927	0.105	0.155	"
<i>g</i>	0.5 "	" "	" "	0.3949	0.07	0.155	"
<i>h</i>	0.25 "	" "	" "	0.2972	0.035	0.155	"
<i>i</i>	0.75 "	0.25 "	" "	0.3949	0.105	0.078	"
<i>j</i>	0.5 "	" "	" "	0.2972	0.07	0.078	"
<i>k</i>	" "	" "	" "	" "	" "	" "	"

Nutrient materials supplied at intervals of 10 days to the cultures of Series A, B, and C, Expt. IV.

TABLE IX B.

Length in  $\mu$  of gametophytes and sporophytes in some cultures of Expt. IV.

Days after the commencement of the Expt.	4	17	22	25	28	31	33
Series A,							
Culture <i>c</i>	8.4 $\mu$					39.2	41.2
Culture <i>d</i>	8.4 $\mu$	12.4 $\mu$	15.8 $\mu$	22.8 $\mu$	32.4	75.6	75.2
						31.9	32.4
						44.4	48.8

The lengths of the sporophytes are given in italics.

in the initial 25 c.c. of sea-water was occasionally sufficient to produce a few sporophytes, Experiment II, Series B, but unless additions were supplied growth was poor.

III. Increase in the additions of phosphate was accompanied by increase in growth. In Experiments II and III, cultures *e* and *f*, supplied with 0.078 mg. P per ten days, showed no filamentous growth until about twenty days later than the cultures supplied with 0.155 mg. (Pl. XXXIX, Figs. 22 and 23).

IV. Supplied with phosphate but no nitrate, growth was thin and filamentous, the gametophytes being very slightly pigmented. Under these conditions one culture alone produced sporophytes (Expt. II, Series B) and growth did not long continue (Pl. XXXIX, Fig. 21).

V. With increased nitrate both the pigmentation and the diameter of the gametophytes increased to some extent, but there was no visible difference in the length of the gametophytes supplied with similar amounts of phosphate but with varying amounts of nitrate.

Thus in light of the same intensity growth depended on the P/N ratio.

Where additions of phosphate were small and additions of nitrate were in excess, growth and development were retarded but sexual reproduction was not inhibited (cultures *e* and *f*).

Where additions of phosphate were greater a slight excess of nitrate did not appear to retard growth to the same extent (culture *c*).

Where additions of phosphate were in excess of nitrate growth was exceedingly filamentous (*g*) and proceeded to some extent at the expense of reproduction.

#### B. *At all concentrations of the medium.*

I. A certain light intensity was necessary for growth and development.

With low light intensities growth and antheridial formation were retarded and but few oogonia developed (Expts. II and III, Series D).

High light intensities did not retard growth and the formation of antheridia, but slightly inhibited the formation of sporophytes (Expt. II, Series B). As the year advanced the optimum light intensity increased. Thus, in Experiment I, set up on January 3, an intensity of 2.5, as measured by the Bee Metre, was most favourable; on January 24 an intensity of 8, and on February 22 an intensity of 16.

### SECTION IV.

#### *The Effect of Different Wave-lengths of Light on Growth and Development.*

In order to exclude different portions of the spectrum, in Experiment I, green and red chambers of celluloid were used, but as they proved to be somewhat unsatisfactory, double bell-jars filled respectively with solutions

of ammoniacal copper sulphate and potassium dichromate were employed in later experiments.

The intensity of the light which reached the cultures was not measured, but the results were sufficiently marked to justify certain conclusions.

The three species of *Laminaria* were used in this section, namely, *L. digitata*, *L. saccharina*, and *L. Cloustoni*, and cultures supplied with equal additions of the combined nutrient solution were set up in daylight, in darkness, and under wave-lengths included in the blue and red portions of the spectrum.

In the first experiment growth was normal in all cultures receiving light, being least rapid in 'red' light, and all male gametophytes bore antheridia. Some sporophytes were formed in the culture exposed to daylight and later in the cultures in the green chamber, but none occurred in the red chamber. No growth took place in darkness beyond the initial stages in germination.

Similar results were obtained from Experiment II with *L. Cloustoni*, with the exception that here sporophytes were produced only in the cultures receiving wave-lengths in the blue part of the spectrum.

In the third series, commenced later in the year, a few sporophytes were produced in daylight and 'blue' light. Antheridia alone occurred in 'red' light, and neither growth nor reproduction occurred in darkness.

Evidence obtained from these three series strongly indicates that wave-lengths in the blue part of the spectrum are almost as favourable in promoting growth and sexual reproduction as daylight in the intensities employed. Under wave-lengths in the red portion growth is rather less, antheridia mature but no oogonia, the female gametophytes being definitely retarded. Neither growth nor reproduction occur in darkness beyond the earliest stages of germination; the germling, however, remains for some months capable of growth on being illuminated.

#### GENERAL DISCUSSION AND CONCLUSIONS.

Many points of discussion arise from the results obtained from these experiments in relation to the work of recent investigators, notably Schreiber.

In the early cultural work on marine algae it was discovered that the addition of nitrate and phosphate to the natural sea-water promoted development, and various solutions containing these salts have been suggested as cultural media. Schreiber found that the sodium salts gave the best results with the diatoms and green algae which he used, and Kylin and Myers used sodium nitrate as a source of nitrogen in the culture of various members of the Laminariaceae. In the present work, however, potassium salts were successfully employed as sources of nutrient material.

Few data are available concerning the proportion in which nitrate and

phosphate are utilized by the marine algae. Schreiber states that while a proportion of 8:1 gives optimum results, a proportion of 5:1 is more convenient for cultural work, since large quantities of nitrate result in such prolific multiplication of bacteria. Myers observed this balance by adding to her cultures a 1 per cent. solution of sodium nitrate and a trace of calcium phosphate.

The first two sections of the present work indicated that almost equal quantities of nitrate and phosphate in the form of potassium salts are utilized by *Laminaria*, but further investigation showed that the balance between the two salts may vary to some extent with the light intensity. The most favourable concentration of phosphorus varied between 0.06 and 0.15 mg. per 25 c.c. sea-water, and nitrogen between 0.035 and 0.105 mg., these additions being supplied at intervals of ten days. The amount of nitrogen as nitrate present in the sea-water at Aberystwyth on March 19, 1930, was 0.007 mg. per 25 c.c.<sup>1</sup>

In consequence of the fact that iodine is present in such large quantities in the higher members of the Phaeophyceae, it is interesting that the minute quantity present in the 25 c.c. of sea-water supplied initially was sufficient for the sexual growth and reproduction of the gametophytes and the production of small plantlets of *Laminaria*. Although periodic additions of 0.0005 grm. per 1000 c.c. accelerated development, larger quantities were ineffectual and high concentrations had an inhibitory effect.

From a comparison of the periods of time taken by the gametophytes to mature in the different experiments and also from Experiments II, III and IV, Section III, it can be seen that light intensity bears a relation to development. There is no fixed optimum light intensity, as it increases as the year advances.

Temperature is also an important factor in that when it is raised above a certain degree it inhibits reproduction though it accelerates growth.

It will be noticed that the growth of the gametophytes in different experiments varied to a great extent. Pl. XXXVII, Figs. 2 and 3, shows that in some cases the female gametophytes increased in size, maintaining their spherical outline, and that prior to the formation of oogonia they assumed an egg-shaped appearance, whereas in other cases the gametophytes became filamentous, and where they were not all fertile increased greatly in length. It was found that the filamentous form was assumed under the following conditions:—

A. Where temperature and light intensity were high.

B. Where the amount of phosphate was in excess of the nitrate.

In these cases the gametophytes were generally sterile. This suggests that where light intensity is high the rate of assimilation is accelerated

<sup>1</sup> Denigès reagent for nitrate determination (7).



and the C/N ratio is too high for the formation of reproductive organs, therefore growth takes place at the expense of development.

In the second case it was found that when the phosphate-nitrate ratio was high growth was rapid, but a high percentage of the gametophytes was sterile.

Growth is proportional to phosphate content in all light intensities. It may therefore be concluded that when the C/N or P/N ratios are high growth proceeds at the expense of development.

Therefore, with suitable light intensity and sufficient phosphate, fertility varies with the nitrate concentration. When phosphate and nitrate are present in sufficient quantity fertility varies with the light intensity.

Very little work has been done on the effect of different wave-lengths of light on the development of the marine algae. Schreiber found that no part of the spectrum was harmful in the culture of phytoplankton, but the present investigation indicates that while the blue part of the spectrum allows of the complete development of the gametophytes, under the red half, not only is growth retarded, but also no oogonia have been observed although antheridia have been produced.

#### SUMMARY.

1. In the present study some of the effects of nutrition and light on the gametophytes of *Laminaria digitata*, *L. saccharina*, and *L. Cloustoni* were analysed experimentally by cultural methods.

2. The zoospores were allowed to settle on glass covers which were subsequently transferred to the experimental Petri dishes containing 25 c.c. of filtered sea-water. They were supplied with small quantities of KI,  $\text{KH}_2\text{PO}_4$ , and  $\text{KNO}_3$ . Cultures were subjected to daylight or to artificial illumination according to the method employed by Schreiber.

3. Series of experiments with adequate controls showed that no growth occurs where the initial supply of sea-water is not renewed and nutrient materials are not supplied.

4. Additions of a combined nutrient solution of KI,  $\text{KH}_2\text{PO}_4$ , and  $\text{KNO}_3$  promote growth and development. They are most effective when supplied in fairly large quantities at intervals of ten days.

5. The amount of iodide present in the initial supply of sea-water allows of the complete development of the gametophytes, but additions of 0.001 mg. of iodide as KI accelerate growth and reproduction.

6. Periodic additions of phosphorus and nitrogen are essential. Increase in the supply of phosphorus produces a corresponding increase in the elongation of the gametophytes. Similar increase in nitrogen concentration does not promote elongation, but produces deeply pigmented gametophytes of greater diameter.

7. Development proceeds most favourably with additions of nitrogen from 0.035 to 0.105 mg. per 25 c.c. of sea-water and with additions of phosphorus from 0.06 to 0.15 mg.

8. Within certain limits higher light intensity and temperature allow of the greater utilization of these salts by the gametophytes. With high P/N and C/N ratios, growth proceeds at the expense of reproduction. Above 16° C. no oogonia are formed.

9. Neither growth nor development occur in darkness, a certain light intensity, varying with the time of the year, being essential for these processes.

10. Gametophytes were subjected to wave-lengths in the different portions of the visible spectrum, and results show that the blue part allows of complete development, but when only the red portion is available, growth and antheridial formation are retarded and oogonial formation definitely inhibited.

This work was carried out under the direction of the late Professor Robinson and was originally intended to form part of a larger work which he had contemplated. Since his death I have compiled the present paper from all the available data. I wish to acknowledge my indebtedness to the Department of Scientific and Industrial Research for financial assistance and to express my gratitude to Professor Newton for her advice and helpful criticism during the preparation of this paper for publication.

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## EXPLANATION OF PLATES XXXVII-XXXIX.

Illustrating Miss Harries' paper on 'An Investigation of Some of the Factors Influencing the Development of the Gametophytes and the Early Stages of the Sporophytes of *Laminaria digitata*, *L. saccharina*, and *L. Cloustoni*'.

### PLATE XXXVII.

Figs. 1-4. Growth stages of *Laminaria saccharina*, Lamour.

Fig. 1. Gametophytes in the 'resting stage'.  $\times 1,000$ .

Fig. 2. Male and female gametophytes.  $\times 1,000$ . Female: spherical or egg-shaped prior to the formation of the oospheres. Male: small, branched, and less deeply pigmented.

Fig. 3. Filamentous gametophytes.  $\times 200$ . Female: long and generally unbranched. Male: much branched and of smaller diameter.

Fig. 4. Sporophytes with primary rhizoids.  $\times 200$ . Note the empty oogonial cases.

Figs. 5-8. Cultures of *L. saccharina* supplied with varying amounts of a combined solution of  $KI$ ,  $KH_2PO_4$ , and  $KNO_3$ , at intervals of 10 days. Photographs taken 94 days after the liberation of the zoospores. Magnification, 100.

Fig. 5. Culture *b'*, supplied with 0.014 mg. N, 0.023 mg. P, and 0.016 mg. I. Gametophytes in early stages of development.

Fig. 6. Culture *c'*, supplied with 0.035 mg. N, 0.06 mg. P, 0.19 mg. I. Young sporophytes with empty oogonial cases and disintegrating male gametophytes.

Fig. 7. Culture *d'*, supplied with 0.07 mg. N, 0.12 mg. P, 0.38 mg. I. Young sporophytes with empty oogonial cases and disintegrating male gametophytes.

#### PLATE XXXVIII.

Fig. 8. Culture *f'*, supplied with 0.14 mg. N, 0.23 mg. P, 0.76 mg. I. Male and female gametophytes showing retarded development.

Figs. 9-11. Photographs of the cultures of the same series as above taken 116 days after the liberation of the zoospores. Magnification, 100.

Fig. 9. Culture *c'*, supplied with 0.35 mg. N, 0.06 mg. P, 0.19 mg. I. Young sporophytes.

Fig. 10. Culture *d'*, supplied with 0.07 mg. N, 0.12 mg. P, 0.38 mg. I. Young sporophytes.

Fig. 11. Culture *e'*, supplied with 0.105 mg. N, 0.18 mg. P, 0.38 mg. I. Large female gametophytes. Small disintegrating male gametophytes.

Figs. 12-14. Series of cultures of *L. saccharina* illustrating the effect of phosphate on growth. (Section II.) Photographs taken 43 days after the liberation of the zoospores. Magnification, 200.

Fig. 12. Culture *b'*, supplied with additions of 0.00031 mg. P. Gametophytes practically undifferentiated.

Fig. 13. Culture *f*, supplied with additions of 0.155 mg. P. Male and female gametophytes showing slightly filamentous growth.

Fig. 14. Culture *i*, supplied with additions of 0.465 mg. P. Female gametophytes very filamentous. Male gametophytes much branched.

Figs. 15-18. Photographs illustrating the effect of nitrate on the growth and development of the gametophytes of *L. saccharina*. (Section II.) Photographs taken 76 days after the liberation of the zoospores. Magnification, 200.

Fig. 15. Culture *b*, supplied with 0.014 mg. N. Male and female gametophytes and an occasional sporophyte.

Fig. 16. Culture *e*, supplied with 0.105 mg. N. Sporophytes well developed.

#### PLATE XXXIX.

Fig. 17. Culture *f*, supplied with 0.14 mg. N. Gametophytes filamentous, sporophytes small.

Fig. 18. Culture *g'*, supplied with 0.21 mg. N. Gametophytes deeply pigmented, showing rather stunted growth.

Figs. 19-24. Photographs further illustrating the effect of phosphate and nitrate on the growth of the gametophytes of *L. saccharina*. Photographs taken 70 days after the liberation of the zoospores. Magnification, 100.

Fig. 19. Control culture in sea-water alone.

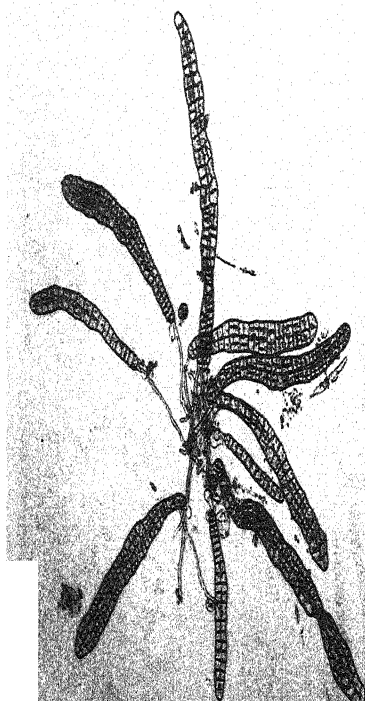
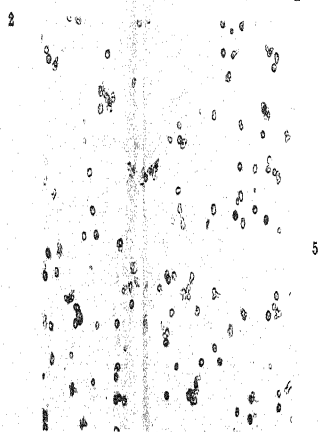
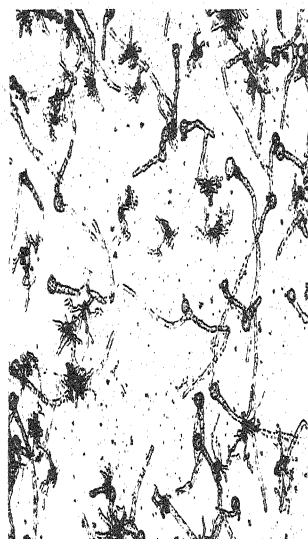
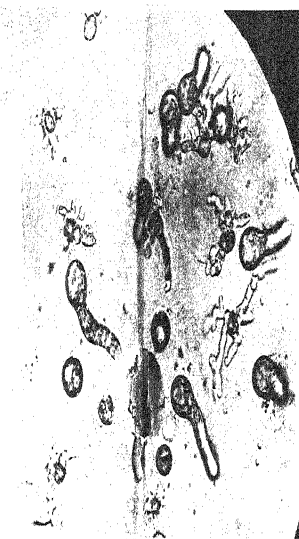
Fig. 20. Culture supplied with 0.07 mg. N per 10 days, and no P. Photographs taken 18 days after the liberation of the zoospores.

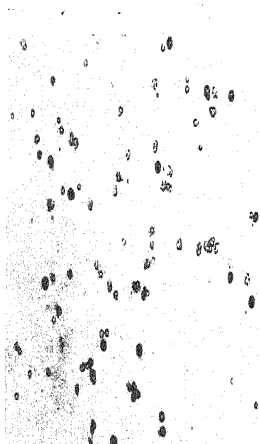
Fig. 21. Culture supplied with 0.155 mg. P per 10 days, and no N.

Fig. 22. Culture supplied with 0.105 mg. N, 0.078 mg. P.

Fig. 23. Culture supplied with 0.14 mg. N, 0.155 mg. P.



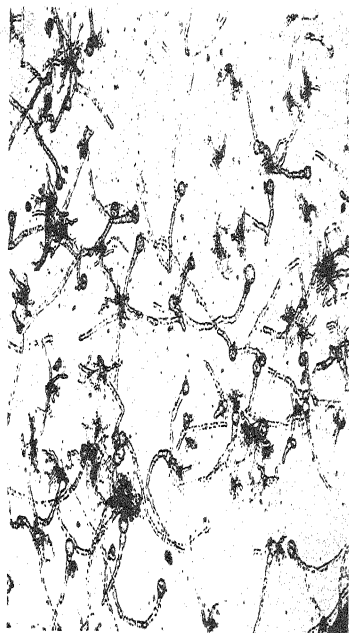




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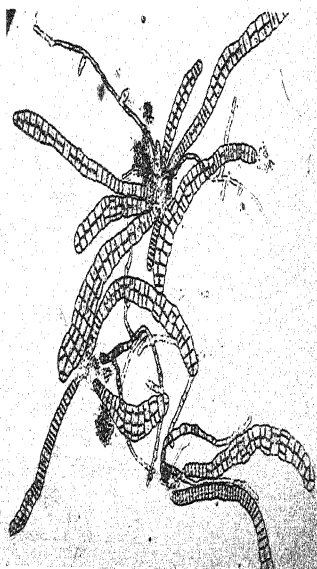
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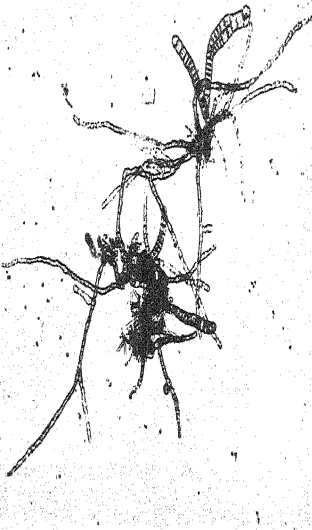
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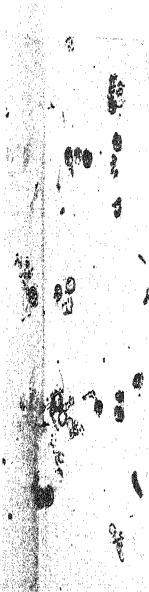
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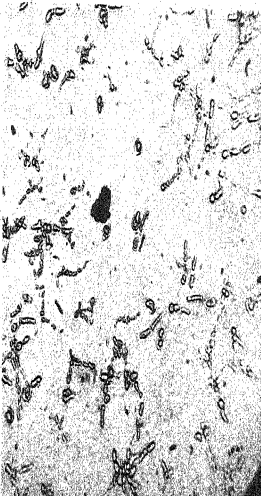
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## Studies in Flower Structure.

### I. On a Peloria of *Digitalis purpurea*, L.<sup>1</sup>

BY

AGNES ARBER.

With six Figures in the Text.

#### I. INTRODUCTION.

THE occurrence of a large, bell- or cup-shaped flower at the apex of the inflorescence in the Foxglove (*Digitalis purpurea*, L.) is an abnormality which has frequently been recorded; the numerous references to the subject in botanical literature have been summarized by Penzig (3, vol. iii, pp. 116-18), and a typical example is illustrated by Worsdell (5, vol. ii, Pl. XXIV). These terminal flowers show a diversity of structure, the most interesting variant being that in which there are several whorls of carpels. So far as I have been able to learn, the only statement relating to this polycyclic type is a short note by the Italian botanist, Massalongo, accompanied by two naked-eye diagrams (2). A re-examination of this remarkable form seemed desirable, and I have recently had the opportunity of making it through the kindness of the Rev. R. H. Edwards, of Netherton Wood, Nailsea, Somerset, who, in June 1928, sent me six peloric Foxgloves from his garden, among which I found one fine example of Massalongo's type of gynaeceum, as well as others showing an approximation to it. The present note records the results of the study of certain features of these peloria by means of serial sections. The structure of the normal Foxglove flower is first considered as a basis for comparison.

#### II. DESCRIPTION.

##### (i) *Normal Structure.*

Figs. 1-3 show the construction of the normal flower of *Digitalis purpurea*, L. In Fig. 1, A<sub>1</sub>-A<sub>5</sub>, p. 930, and Fig. 2, A<sub>6</sub>-A<sub>9</sub>, p. 931, a series of transverse sections may be followed from below upwards through a single

<sup>1</sup> This paper represents part of the work carried out with the aid of a grant from the Dixon Fund of the University of London.

flower-bud.  $A_1$  and  $A_2$  show the base of the flower and the detachment of the calyx. The stamen whose bundle is marked with an asterisk in  $A_4$ ,

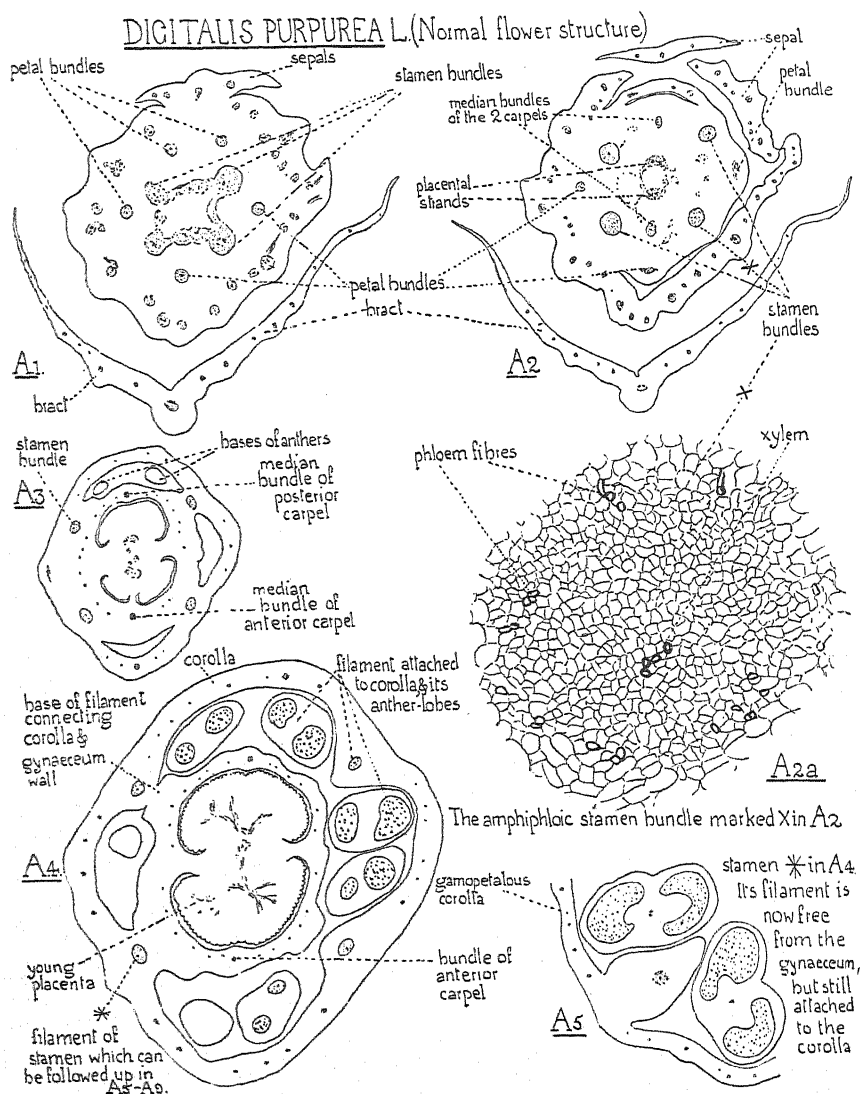


FIG. 1. *Digitalis purpurea*, L. Structure of normal flower, garden material.  $A_1$ – $A_5$  (and  $A_6$ – $A_9$ , p. 931), transverse sections from a series from below upwards through a flower-bud;  $A_1$ – $A_3$  ( $\times 16$  circa);  $A_2$  a ( $\times 218$  circa);  $A_4$  and  $A_5$  ( $\times 26$  circa). In  $A_2$  the outermost sepal was broken and is slightly reconstructed.  $A_2$  a, the stamen bundle  $\times$  in  $A_2$ , more highly magnified. In  $A_3$  and  $A_4$ , bract and calyx omitted. The series through the stamen marked \* in  $A_4$  is continued in  $A_5$  and in Fig. 2, p. 931.

is traced upwards in  $A_5$  and in Fig. 2,  $A_6$ – $A_9$ , p. 931. In  $A_7$  it is noticeable that not only the filament, but each anther-lobe, has a vascular bundle.

The septum between the two pollen-sacs of each lobe is remarkably massive, and protrudes into the sac, so that the archesporium is kidney-shaped in

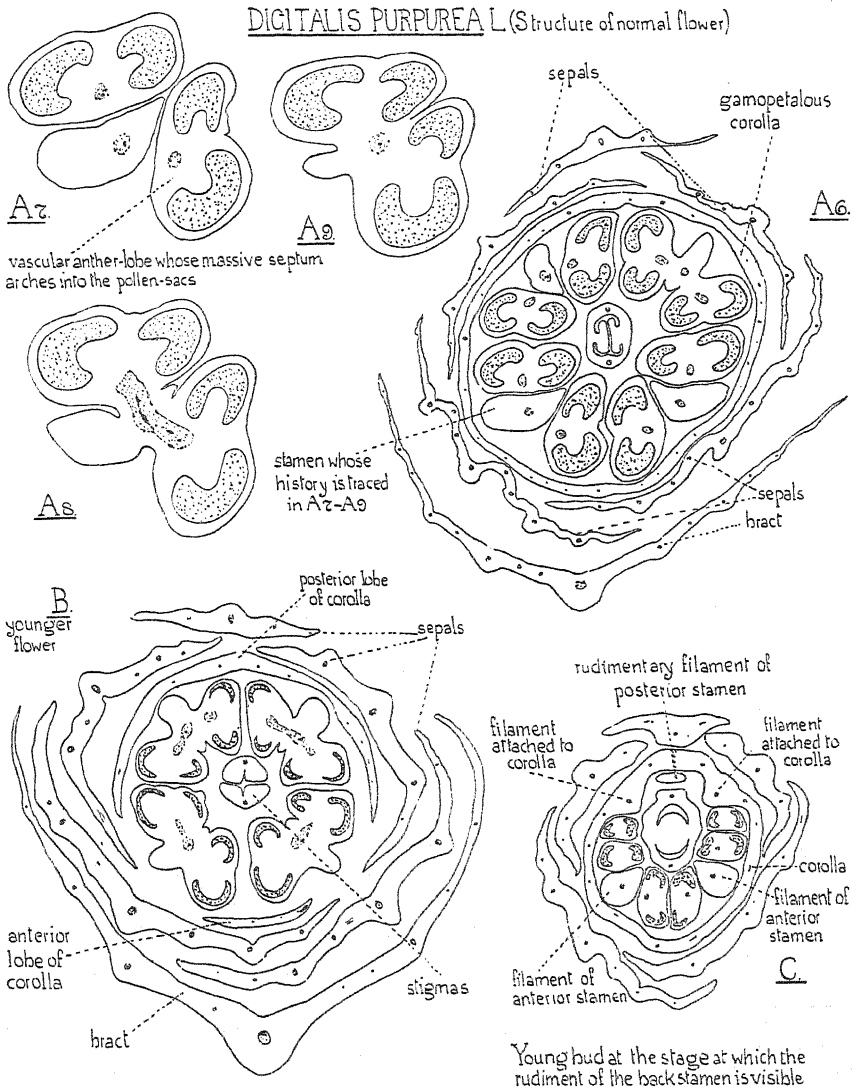


FIG. 2. *Digitalis purpurea*, L. Structure of normal flower, garden material. A<sub>6</sub>-A<sub>9</sub>, continuation of series drawn in Fig. 1, p. 930, A<sub>6</sub> ( $\times 16$  circa), A<sub>7</sub>-A<sub>9</sub> ( $\times 26$  circa). B, transverse section of another younger flower-bud ( $\times 26$  circa), cut through the level of stigmas and anthers. A rudiment of the missing posterior stamen was visible at a lower level. C, transverse section through a flower-bud younger than B ( $\times 26$  circa) to show rudimentary filament of the missing posterior stamen.

section. This feature is shown still more strikingly in Fig. 2, B, which represents a younger flower; the septa are exaggerated to such a degree

that the pollen-sacs are reduced to mere chinks.  $A_8$ , which is cut at a higher level, shows the attachment of the anther-lobes to the filament. The two bundles of the anther-lobes in  $A_7$  are here seen to be branches

DIGITALIS PURPUREA L. (Structure of a very young, normal flower-bud)

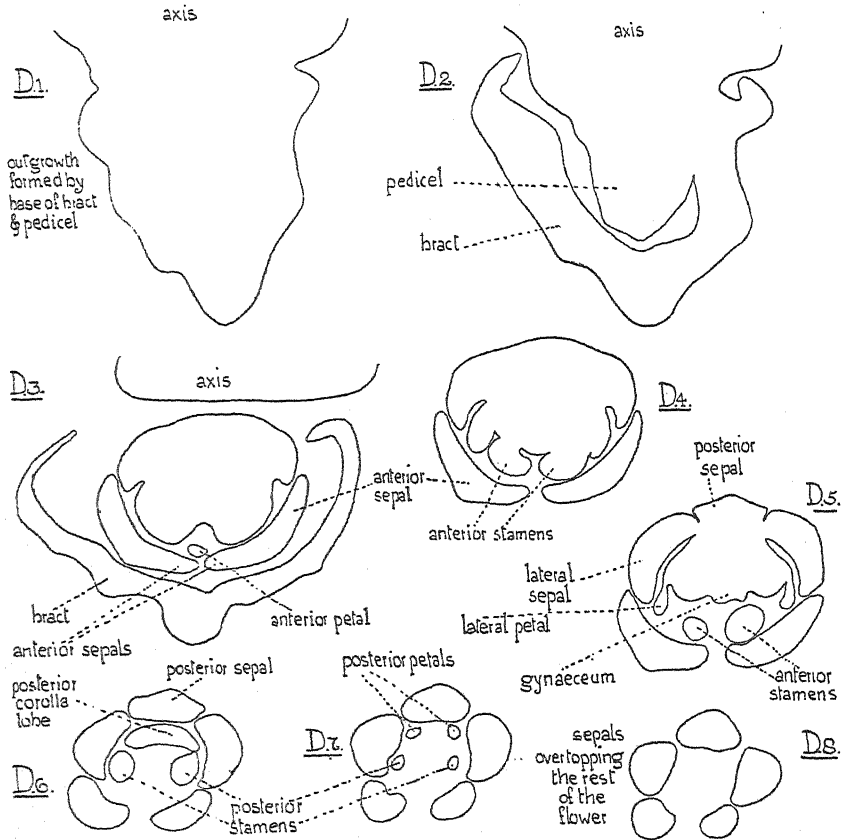


FIG. 3. *Digitalis purpurea*, L. Structure of normal flower, garden material.  $D_1$ – $D_8$ , sections from a transverse series from below upwards through a flower-bud younger than those drawn in Figs. 1 and 2 ( $\times 53$  circa). In  $D_5$  the extremely rudimentary gynaecium can just be distinguished on the anterior face of the receptacle.

from the filament bundle, which have dipped down into the lobes.  $A_9$  is cut above the anther-lobe bundles. The filament bundle is amphiphloic at the level of Fig. 1,  $A_2$ . Its structure is shown in detail in  $A_{2a}$ ; it has a few lignified xylem elements in the centre, while scattered groups of phloem fibres are arranged round the periphery. In  $A_7$  all three strands tend to be amphiphloic, and the filament bundle is still of this type at the level of  $A_9$ , but it becomes collateral before it dies out. In the A series

there was no external trace of a posterior stamen, nor was there any vascular strand destined for it; but it can be seen in Fig. 2, C, p. 931, which is a section of a younger flower. It consisted of a minute trace of a filament in the median position, between the back of the gynaeceum and the corolla; there was no bundle corresponding to it. In a series of sections passing through the tip of an inflorescence I saw such a rudiment in five successive flower-buds. The bud drawn—which was 1.5 to 2 mm. in diameter—was the one in which it was most clearly visible. In older flowers the rudiment could not be recognized, while, on the other hand, the buds of the younger series, which succeeded the five buds in question were too immature to show it. This rudiment is probably variable in its occurrence, since von Wettstein's mention of it in the genus (4) suggests that it may be found in the mature flower.

Fig. 3, p. 932, is from a series passing through a flower-bud, even younger than the buds from which the sections drawn in Figs. 1 and 2 were cut.  $D_1$  and  $D_2$  indicate the massiveness of the enclosing bract in relation to the flower rudiment. It is probable that the pressure conditions set up between bract and axis in the earliest stages have some connexion with the zygomorphy of the flower.

It is, however, the structure of the gynaeceum which chiefly concerns us now, for comparison with that of the peloric forms illustrated in Figs. 4-6. In Fig. 1,  $A_2$ , which shows the receptacle below the gynaeceum, the median bundles of the two carpels are seen to north and south, with their less well-defined marginal strands lying between them. In  $A_3$  the front placenta shows its bifid character; it dips into two cavities in the base of the ovary. The back placenta shows the same feature at a lower level. Towards the top of the ovary the placental region of each carpel dissociates completely into the two margins from which it is formed, though these margins remain in union with the corresponding regions of the opposed carpel (Fig. 2,  $A_6$ ).

#### (ii) *Peloric Structure.*

In the first of the terminal peloric flowers received from Mr. Edwards, the corolla was preceded by an irregular crowd of bract-like structures, some of which were partially petaloid; in this medley it was impossible to distinguish bracts from sepals. Some of these structures had reduced corollas in their axils. Above them there were about nineteen corolla lobes, followed by fifteen stamens—two petaloid and thirteen normal. The gynaeceum was the best example of Massalongo's type which I have seen; it is illustrated in Fig. 4, p. 934. Its external appearance is shown in  $A_{10}$ , at about four-fifths of its natural size.  $A_1$  is a segment of a section of the basal region of the ovary. There is an outer whorl of carpels which are cut through their placentae, and a second interior series cut through their

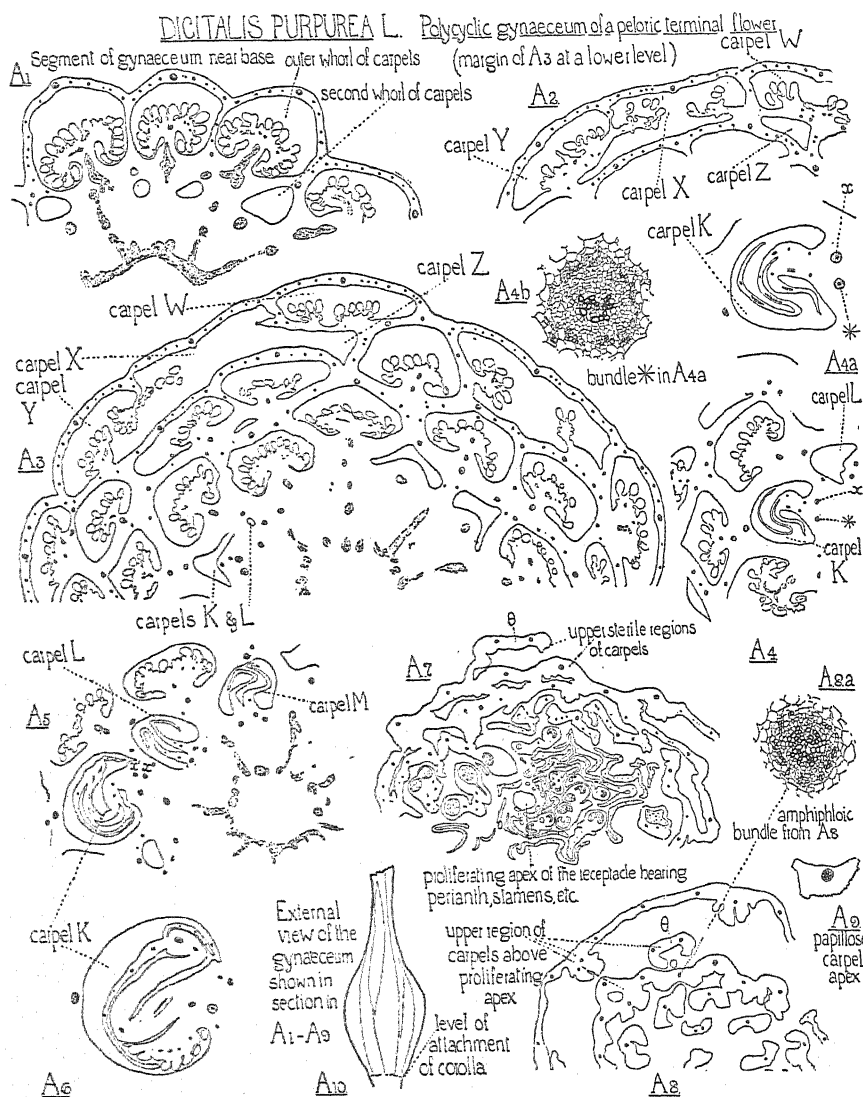


FIG. 4. *Digitalis purpurea*, L. Gynaecium structure of first peloric flower, Nailsea, June 1928. The gynaecium as a whole is shown in A<sub>10</sub> ( $\times 4/5$  circa). A<sub>1</sub>-A<sub>9</sub> are from transverse sections from below upwards through this gynaecium, and the proliferation internal to it. Owing to its size, it had to be cut into segments before microtoming, with the result that the orientation is not consistent, except within the series A<sub>2</sub>-A<sub>9</sub>, and between the two sections A<sub>7</sub> and A<sub>8</sub>. A<sub>1</sub> ( $\times$  nearly 8), part of a section near the base of the ovary. A<sub>2</sub>-A<sub>9</sub>, series through a part of the gynaecium, above A<sub>1</sub> ( $\times$  nearly 8, except A<sub>4a</sub> and A<sub>9</sub>,  $\times 13$  circa, and A<sub>4b</sub>,  $\times 109$  circa). The carpels K, L, X, Y, Z are lettered in order that their changes of structure in passing up may be followed more easily. A<sub>7</sub> and A<sub>8</sub>, higher in the gynaecium, above the level of the ovary ( $\times$  nearly 8). The relation of these two diagrams can be understood from the position of the letter  $\theta$ , which marks a corresponding point in both; the outermost carpillary wall is omitted in A<sub>7</sub>, but shown in A<sub>8</sub>. A<sub>8a</sub>, median bundle of carpel ( $\times 109$  circa). A<sub>9</sub>, one of the carpels near the tip of the gynaecium ( $\times 13$  circa).

empty basal regions.  $A_2-A_6$  is a series through a higher part of the gynaecium than that shown in  $A_1$ ; this series indicates the changes which

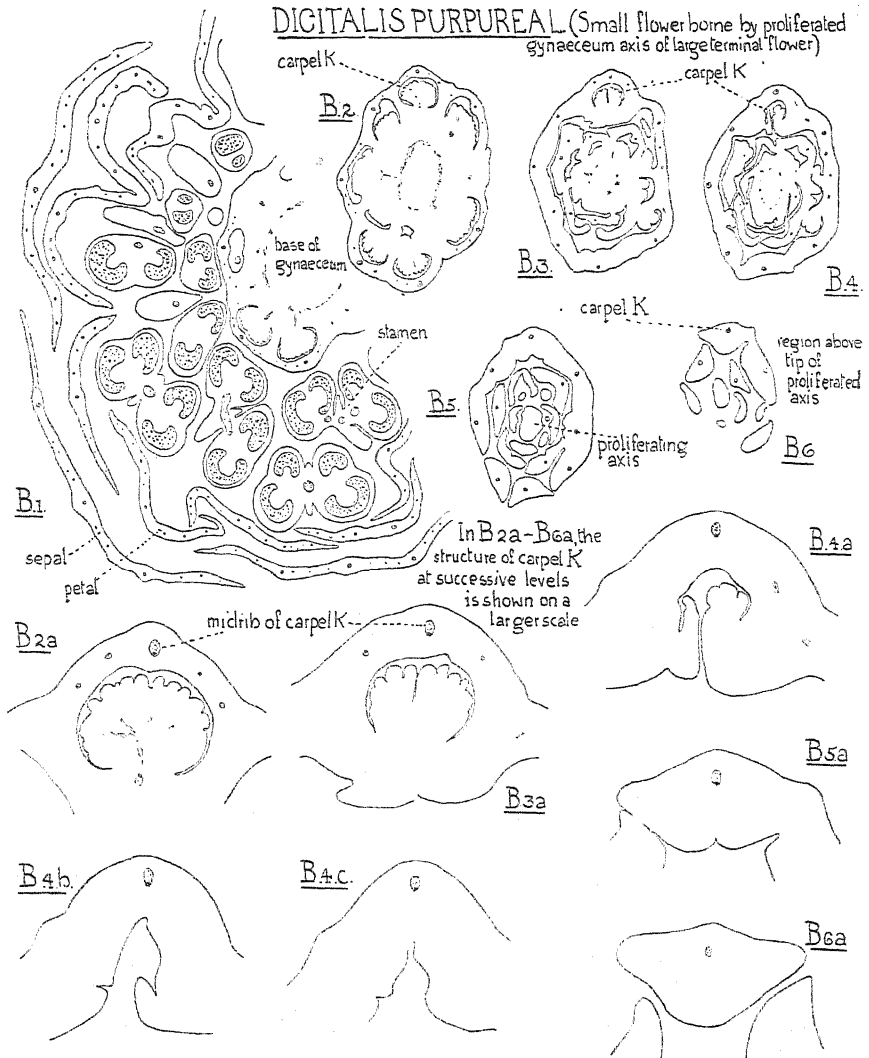


FIG. 5. *Digitalis purpurea*, L. Structure of a small apical flower from the second perianth. Nailsea, 1928.  $B_1-B_6$ , sections from a transverse series from below upwards through the flower ( $\times 15$  circa); except in  $B_1$  the gynaecium alone is shown.  $B_2a-B_6a$ , larger diagrams ( $\times 53$  circa) in which carpel K in  $B_2-B_6$  can be followed more easily.  $B_2a, B_3a, B_4a$ , levels of  $B_2, B_3$ , and  $B_4$ ;  $B_4b, B_4c$ , levels between  $B_4$  and  $B_5$ ;  $B_5a$ , between  $B_5$  and  $B_6$ ;  $B_6a$ , above  $B_6$ .

occur in the placentae of the individual carpels between their basal and distal regions.  $A_3$  is a section of half the ovary, giving a general idea of its configuration. It will be seen that the carpels form four concentric series, whereas Massalongo's specimen showed three only. It is probable



that these series represent four whorls, but they are somewhat irregular, and it would perhaps be possible to interpret the carpels as spirally arranged. Three of the outer carpels (X, Y, Z) form one cavity in  $A_3$ , but

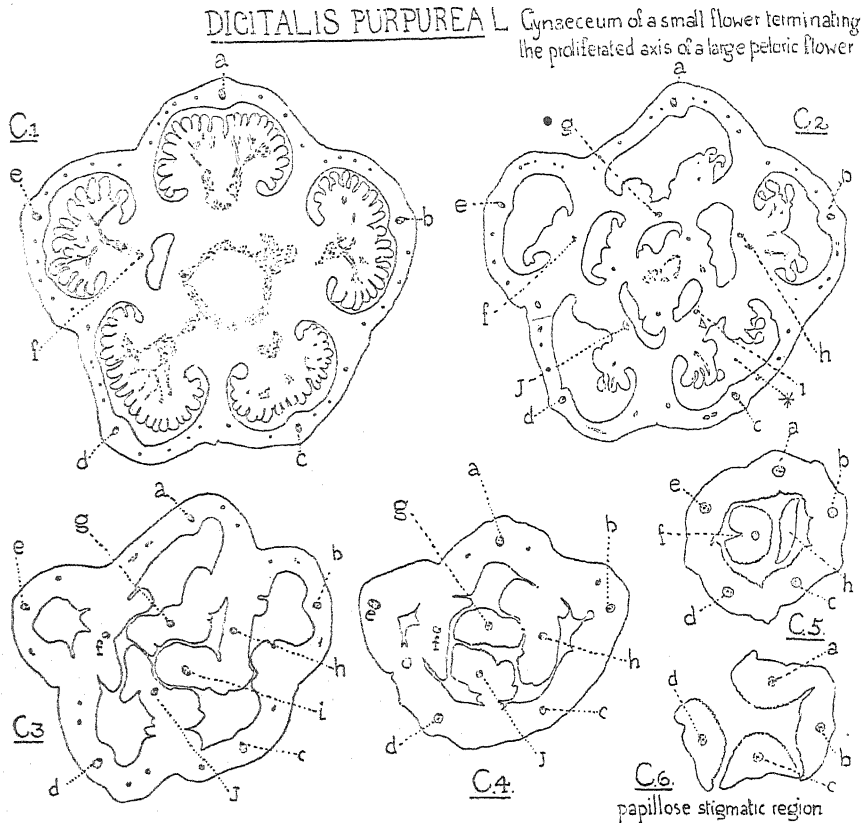


FIG. 6. *Digitalis purpurea*, L. Structure of small apical flower from the third peloria, Nailsea, 1928. C<sub>1</sub>-C<sub>6</sub>, transverse series from below upwards through the gynaecium ( $\times 26$  circa). The five fully developed outer carpels are lettered a-e, and the five inner carpels f-j.

$A_2$  shows that at a lower level they are separate. The history of two of the higher carpels, K and L, can be traced in  $A_3$  to  $A_6$ ; the duplex character of the placenta is clearly defined, especially in  $A_4a$ . In  $A_6$ , one of the margins of carpel K is bearing ovules, while the other has suffered an abnormal leafy development.  $A_7$  and  $A_8$  are sections higher in the gynaecium, above the ovary region. The relation of these two diagrams can be understood from the position of the letter  $\theta$ , which marks a corresponding point in both; in  $A_7$  the outermost carpellary wall is omitted. In this section the flattened upper regions of the carpels are still more or less fused with one another. This section shows a feature which is common to the six pelorias examined—the proliferation of the axis. The uppermost

carpels are succeeded by a few abortive stamens, and then a series of perianth members, followed by more stamens, in which malformations are frequent. Above these, the axis produces some rudimentary leaf members, and then dies out. This proliferated tip of the axis was entirely enclosed within the gynaeceum, and its existence was not suspected until the gynaeceum was sectioned.  $A_8$  is cut at a level above the tip of the proliferating axis, and thus shows only the carpels, which are now separating and revealing their one-bundled character. The median bundles of the carpels are bicollateral below, but at the level of  $A_8$  they are all amphiphloic (cf.  $A_8a$ ). The two amphiphloic bundles,  $x'$  and  $x''$  in  $A_5$ , have arisen by division of the amphiphloic marginal bundle of carpel K, marked  $x$  in  $A_4$  and  $A_4a$ .  $A_9$  represents one of the carpels near the tip of the gynaeceum, to show the papillose character of its inner surface.

A second peloric terminal flower from Nailsea was cup-shaped, and had nineteen stamens. The stamens were followed by a gynaeceum resembling that shown in Fig. 4,  $A_{10}$ , but the proliferation of the floral axis was carried further. It was prolonged as a leafy shoot terminating in a small flower, whose structure is illustrated in Fig. 5, p. 935.  $B_1$  shows part of a transverse section through this flower. The petals are free for most of their length, but at, or just below, the base of the gynaeceum they are connected with one another, and with the stamen bases and the receptacle. Except in  $B_1$  the gynaeceum alone is shown. The outer whorl consists of about seven ovule-bearing carpels ( $B_2$ ). These are succeeded by a number of leafy structures borne by the proliferated axis of the gynaeceum ( $B_3$  and  $B_6$ ). In  $B_2a$  to  $B_6a$  carpel K is traced from below upwards.

A third inflorescence from Nailsea terminated in a flower with a large irregular cup-shaped corolla, with inner lobes that may have been staminal. There were twelve stamens. In place of a gynaeceum the axis emerged in the centre of the peloria, bearing a number of bracts, some green and some partially petaloid; it terminated in a small flower whose gynaeceum is shown in a series of sections in Fig. 6, p. 936; in these sections the median bundles of the carpels are lettered  $a-j$ . In  $C_1$  the five fully developed outer carpels ( $a-e$ ) are seen, and one of the inner carpels ( $f$ ). In  $C_2$  the cavities of the five inner carpels ( $f-j$ ) have come into view. These are succeeded by a few more leafy structures, above which the axis dies out.  $C_3$  is cut above the tip of the axis, so that only the carpels are visible. In  $C_5$  the five outer carpels are united by their margins, forming a continuous ring. Four of the five outer carpels, but none of the inner carpels, reach to the level of  $C_6$ .

### III. DISCUSSION.

I will not now attempt to discuss the meaning of peloric organization in general, since this is a subject which I hope to review in a later paper.

I will only deal here with the carpellary structure of the normal and peloric foxgloves described in the earlier part of this article. In recent years doubt has been cast by more than one writer upon de Candolle's<sup>1</sup> interpretation of the Angiospermic carpel as a leaf-member bearing ovules marginally. It must be confessed that this interpretation has often been accepted by botanists rather as an article of faith than as a conclusion drawn from definite evidence, and those who have questioned it have done good service in making it evident that the reasons underlying it need reassessment. I think that the comparison of normal and polycyclic gynaecea of *Digitalis purpurea* throws some light upon the nature of the carpel, so I will briefly consider these gynaecea from this point of view.

On the Candollean interpretation the normal gynaeceum of the foxglove consists of two carpellary leaves facing one another, with their midribs in the anteroposterior plane, and bearing ovules on their incurved margins—the margins of each carpellary leaf being fused with one another, and the two fused marginal pairs being united back to back. But, with fusion carried so far, it becomes difficult to demonstrate the correctness of this interpretation. However, the structure of the uppermost region of the ovary confirms it in part. For at the level shown in Fig. 2, A<sub>6</sub>, p. 931, the margins of each carpel are free from one another, though united to those of its neighbour. For more complete evidence on the same lines, we must turn to the peloric gynaeceum drawn in Fig. 5, p. 935. Here the outer carpels—not being hampered, as in the normal bicarpellary foxglove, by a congenital face-to-face union with another carpel—show their construction less ambiguously. One of these carpels is traced from below upwards in Fig. 5, B<sub>2</sub>a to B<sub>6</sub>a. This series is best followed downwards from B<sub>6</sub>a, which shows the carpel in its apical region as a single, free, leaf-member. Its apex is solid rather than flattened, thus recalling the thickened tips of many foliage leaves. In B<sub>3</sub>a it is becoming attached to its neighbours on either hand, but not by its margins, which are separated by a groove in the median line; the attachment takes place by the dorsal surface, between the midrib and the margin. In B<sub>4</sub>b, the carpel margins are developing, and in B<sub>4</sub>a we see that they are ovule-bearing. They are still free from one another at this level, but in B<sub>3</sub>a they have fused, although a cleft in the placenta still reveals its dual character. By the time we reach B<sub>2</sub>a this duality can no longer be detected.

Though these lower carpels of the polycyclic gynaecea do not individually diverge much in structure from those of the normal type, the higher carpels are apt to be more aberrant, and to reveal their foliar nature more clearly. As examples in which the placental region obviously consists of associated leaf-margins, we may point to carpel K in Fig. 4, p. 936, A<sub>4</sub>

<sup>1</sup> A. P. de Candolle was not the only botanist to arrive at this theory, but it seems best to associate it with his name, since he was the first to give it full and clear expression.

and  $A_4\alpha$ , and carpels L and M in  $A_5$ . And apart from the detailed structure of the individual carpels, the polycyclic gynaecium, considered as a whole, throws light upon the nature of the carpel. For the multiplication of the gynaecium units, and their correlated rearrangement, makes it possible to check the Candollean conception more completely than in the normal gynaecium. It will be seen at once from such diagrams as Fig. 4,  $A_5$ , that the *unit* which has multiplied to give the polycyclic gynaecium is none other than the carpel in the Candollean sense. For on any other theory we should be forced to make the unworkable assumption of the multiplication and dissociation of units consisting, not of individual leaves, but of complexes in which fractions of leaves, entirely divorced from their fellow fractions, play a part.

We may conclude that (i) the individual construction of the units of which the normal and the polycyclic gynaecium are made up, and (ii) the relation which these units bear to one another in the polycyclic gynaecium, afford definite confirmation of de Candolle's theory of the carpel.

#### IV. SUMMARY.

The structure of the normal flower of *Digitalis purpurea*, L., is described (pp. 929-31 and Figs. 1-3) for comparison with the peloric flowers which form the main subject of the paper. Certain peloric flowers showing Massalongo's polycyclic type of gynaecium (2) are then described (pp. 933-7 and Figs. 4-6). It is shown (pp. 937-9) that in these gynaecia the morphological structure is more completely revealed than in the bicarpellary type. Their study confirms the Candollean view that the carpel is equivalent to a leaf whose marginal regions are ovuliferous—a view which certain recent writers have proposed to discard in favour of other interpretations.

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# The Determination of Fossil Angiosperms by the Characteristics of their Vegetative Organs.

BY

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With six Figures in the Text.

## INTRODUCTION.

HOW can fossil Angiosperms be accurately determined by means of their vegetative organs alone?

The old method of diagnosis, consisting in comparing the *form* and *venation* of the fossil organ with similar characters in a comparable living organ, a method used by Bowerbank, Ettingshausen, Gardner, De La Harpe, Heer, and by many others, has still a few supporters (Berry, Knowlton, and others), but generally speaking, it has been abandoned. Determinations founded solely on form and venation must surely be regarded as based on inadequate evidence.

The new method of diagnosis, and the one chiefly in use at the present day, consists in the study of the *form* and *venation* of the fossil organ combined with that of the characters of its *epidermis*. It has appeared to several botanists that by comparing these three characters with similar features in a living plant, accurate determinations can be ensured. Mention may here be made of the following fossil Angiospermous foliage that has been named by utilizing this method:

*Dicotylophyllum Stopesii*, Bandulska (7) (afterwards corrected to *Stopesae* (8)), later referred to *Nothofagus*, see Bandulska (8).

*Dicotylophyllum spiculatum*, Bandulska (7), later referred to *Aniba*, see Bandulska (9).

*Dicotylophyllum sinuatum*, Bandulska (7), later referred to *Rhodomyrtus*, see Bandulska (11).

*Fagus bournensis*, Bandulska (8).

*Neolitsea Gardneri*, Bandulska (9).

*Litsea Edwardsi*, Bandulska (9).

*Litsea hirsuta*, Bandulska (9).

*Litsea bournensis*, Bandulska (9).

*Lindera cinnamomifolia*, Bandulska (9).

*Lindera cinnamomifolia*, var. *porifera*, Bandulska (9).

*Lindera Batheri*, Bandulska (9).

*Cinnamomum Wonnacotti*, Bandulska (10).

*Tristania bournensis*, Bandulska (11).

All these Angiosperms are from the Bournemouth Freshwater beds in the Bournemouth vicinity of the Hampshire Basin, and have been carefully described by Bandulska (7, 8, 9, 10, 11).

With the above results at hand, it was decided to apply the same new method of diagnosis to the still older Eocene plants from the Pipe-Clay horizon, in the same area, but immediately to the west of the Bournemouth Freshwater Beds, around Poole Harbour, Dorset. It was hoped that the determinations made by Gardner and others (based on the old method) would be fully confirmed.

The clay-pits immediately around Poole Harbour were searched for plant-remains during the years 1923 to 1926. The collecting was unfortunate. Where good form and venation were impressed on the clay, the epidermis was missing; when epidermis occurred it was always in a very fragmentary condition, with no indication of the plant-organ to which it had been originally attached.

Since the impressions gave insufficient data for diagnosis, work was concentrated on the fragments of epidermis. With no clue as to the original plant-organ, the selection of living epidermis for comparison with the fossil epidermis had to be both varied and extensive, and some 170 species were examined. (For genera investigated see pages 944 and 945).

The first selection of living epidermis was based on Gardner's records of plants from the Bagshot Beds around Poole Harbour. This was followed by reference to Bandulska's (7), (8), (9), (10), (11) determinations in the adjoining area of Bournemouth Freshwater Beds. Later, reference was made to work on the plant-remains in the Eocene Beds of North America, and for this purpose the publications of E. W. Berry, W. Bullock Clark, J. W. Dawson, F. H. Knowlton, and D. P. Penhallow were consulted. Additional sources of information included the writings of W. Branwhite Clarke, H. Dobell, J. C. Mansel-Pleydell, W. T. Ord, Louis H. Ruegg, O. White, and others.

The extensive examination of living epidermis gave no help in the determination of the fragments of fossil epidermis. Indeed, as regards Angiosperms, the investigation revealed so great a lack of characteristic features in the epidermis that the value of the epidermis, even combined with the external characters of form and venation, seemed very doubtful.

In consequence of this conclusion, it was decided to carry out a critical examination of the modern method of combining form and venation with

the characters of the epidermis in the determination of the vegetative parts of fossil Angiosperms.

External form alone, venation considered alone, or form combined with venation, in accordance with the results of other observers, were found insufficient for the determination of Angiosperms.

The diagnostic value of the epidermis alone, however, was uncertain. Epidermal features that seemed diagnostically weak were in constant use as strong characters in modern determinative work. The use of such features as the following may be quoted in support of this statement: (1) The shape of the epidermal cell. (2) The size of the epidermal cell. (3) The outline or sinuation of the lateral wall of the epidermal cell. (4) The subsidiary cells bordering the stoma. (5) The level of the stoma in the epidermal tissue. (6) The number of stomata per unit area. (7) The arrangement of stomata. (8) The size of the stoma. (9) The structure of the stoma.

Features (4) and (9) have taken the foremost places in the determinations of the Angiosperms of Bournemouth Freshwater age (see Bandulska (7), (8), (9), (10), (11)). But the absence of subsidiary cells in as many as 153 families of Angiosperms (as recorded by Solereder (68)), and the similarity in the structural design of the stoma in many of these families seemed to show that the presence or absence of subsidiary cells and the nature of the stomatal structure could not satisfactorily be utilized in determinative work.

This paper sets forth a critical examination of the epidermis of not less than 170 species of Angiosperms, and demonstrates that every character of the Angiospermous epidermis must be considered diagnostically weak.

And further, the examination suggested that stomatal structure (the generally considered strongest determinative feature of the epidermis) even combined with form and venation could not be regarded as of value in the diagnosis of the vegetative organs of Angiosperms. In order to decide this question, four of the 153 families enumerated by Solereder as having no subsidiary cells to the stoma were selected for study. These were Campanulaceae, Compositae, Malvaceae, and Scrophulariaceae.

It was comparatively easy to show that *stems* from plants belonging to different families agreed with one another in form, venation, and stomatal structure. The simplicity of form and venation in many of the *foliage leaves* of these families suggested a possibility that foliage leaves agreeing in form, venation, and stomatal structure could also occur in widely separated families.

It is interesting that in four cases, foliage leaves with the same form, venation, and stomatal structure (viewed on the surface and in vertical section) were found in different families.

This result must surely be considered of importance to the fossil



botanist concerned with the diagnosis of the *vegetative parts* of fossil Angiosperms. Not only does it place much of the present-day naming of these vegetative organs in a very precarious position ; but it emphasizes (if emphasis is needed) the importance of the *fruits* and *seeds* of fossil Angiosperms, for these organs are of indisputable diagnostic value.

*The GENERA INVESTIGATED from the Point of View of Vegetative Characteristics.*

Family.	Genus.
Aceraceae . . .	<i>Acer</i>
Apocynaceae . . .	{ <i>Ansonia</i> <i>Rhazya</i>
Araliaceae . . .	{ <i>Aralia</i> <i>Pseudopanax</i>
Asclepiadeae . . .	<i>Asclepias</i>
Betulaceae . . .	<i>Betula</i>
Campanulaceae . . .	{ <i>Campanula</i> <i>Lobelia</i>
	<i>Actinomeris</i>
	<i>Aster</i>
	<i>Boltonia</i>
	<i>Erigeron</i>
	<i>Gaillardia</i>
Compositae . . .	{ <i>Gymnolomia</i> <i>Helenium</i> <i>Helianthus</i> <i>Helichrysum</i> <i>Inula</i> <i>Solidago</i>
	{ <i>Fagus</i>
Cupuliflorae . . .	{ <i>Quercus</i>
	{ <i>Armeria</i>
Gentianaceae . . .	{ <i>Gentiana</i>
Hippocastanaceae . . .	<i>Esculus</i>
	<i>Mentha</i>
	<i>Nepeta</i>
Labiatae . . .	{ <i>Prunella</i> <i>Salvia</i> <i>Stachys</i> <i>Cinnamomum</i>
	<i>Laurus</i>
Lauraceae . . .	{ <i>Litsaea</i> <i>Persea</i> <i>Sassafras</i>
	{ <i>Cytisus</i>
Leguminosae . . .	{ <i>Lathyrus</i>
	{ <i>Althaea</i>
Malvaceae . . .	{ <i>Kitaibelia</i> <i>Lavatera</i> <i>Malva</i>
Myricaceae . . .	<i>Myrica</i>
	<i>Anagallis</i>
Primulaceae . . .	{ <i>Cyclamen</i> <i>Lysimachia</i> <i>Primula</i>
Proteaceae . . .	{ <i>Banksia</i> <i>Protea</i>

Family.	Genus.
Ranunculaceae . . .	<i>Aconitum</i>
	<i>Anemone</i>
	<i>Aquilegia</i>
	<i>Caltha</i>
	<i>Clematis</i>
	<i>Delphinium</i>
	<i>Paeonia</i>
	<i>Ranunculus</i>
Salicaceae . . .	<i>Populus</i>
	<i>Salix</i>
	<i>Antirrhinum</i>
Scrophulariaceae . . .	<i>Bartsia</i>
	<i>Celsia</i>
	<i>Digitalis</i>
	<i>Euphrasia</i>
	<i>Linaria</i>
	<i>Mimulus</i>
	<i>Pedicularis</i>
	<i>Scrophularia</i>
Ulmaceae . . .	<i>Ulmus</i>
Urticaceae . . .	<i>Ficus</i>

Amaryllidaceae . . .	<i>Galanthus</i>
	<i>Narcissus</i>
	<i>Crocus</i>
Iridaceae . . .	<i>Gladiolus</i>
	<i>Iris</i>
	<i>Sisyrinchium</i>
	<i>Allium</i>
Liliaceae . . .	<i>Convallaria</i>
	<i>Polygonatum</i>
	<i>Ruscus</i>
	<i>Scilla</i>
	<i>Tulipa</i>
Palmae . . .	<i>Sabal</i>

*The Technique used in the Mounting of the Epidermis.*

In consequence of the results obtained by Sahni and Singh (62) in the mounting of the epidermis of *Fitzroya patagonica* (Hook. fil.), a brief note on the technique used in the mounting of all the epidermis (fossil and living) for this paper must be inserted here.

The method sketched below has been employed for both the fossil and the living epidermis, hence the criticisms of method made by these writers have no bearing on the results set forth in this paper.

For clearing, the epidermis has been submerged in nitric acid (the strength of the acid varying according to the texture of the epidermis), and the liquid slowly brought to the boiling point.

For staining, immersion of the epidermis in a mordant (picric acid) at

the outset has been found essential. This has been followed by treatment with safranin in alcohol. The epidermis has then been dehydrated in absolute alcohol, cleared in origanum oil, and mounted in Canada balsam.

*A Critical Examination of the EPIDERMIS of the Vegetative Organs of Angiosperms.*

*A. The value of external glands.*

Among the Angiosperms examined (see above list), the arrangement of glandular hairs has been found always to be irregular. The number per unit area in comparable regions of different plants has also been of no diagnostic value in any family, genus, or species.

According to Solereder (68) only two structures are exhibited in the external glands of Angiosperms, (1) 'small forms . . . which have a simple structure', and (2) those 'which are mostly of large size and generally have a complicated structure'; but the writer adds, 'it must, however, be pointed out that the boundary-line between the two kinds of hairs is artificial and therefore not sharply marked'. Many families are recorded by this writer as possessing both kinds of structure. Moreover, in eighty families 'external glands have not been observed in any form'. These observations have been confirmed in the genera mentioned above.

Thus, it has seemed evident that *external glandular hairs* cannot be used for determining the vegetative parts of Angiospermous plants.

*B. The value of clothing hairs.*

To use Solereder's (68) definition, 'clothing and glandular hairs are distinguished from one another by the absence and presence respectively of secretion.'

The arrangement of clothing hairs in the epidermis of the Angiosperms examined (see above list) has been found always to be inconstant, and (as is commonly known) there has been marked frequency over veins. Their number per unit area in comparable regions of any particular plant-organ in a family, genus, or species has been found to be variable. Hence, neither the arrangement nor the number per unit area have been of diagnostic value. These observations are in accordance with Stober's (69) investigations.

Zalenski (quoted by Maximov (54)) reports on the increasing number of clothing hairs per unit area in passing from the lower to the higher levels of the plant; Maximov (54), Stober (69), Yapp (81) find generally, and Salisbury (quoted by Yapp (81)) in the case of the 'wild Raspberry', that there is an increase in the number of clothing hairs per unit area in passing from the basal to the apical leaves of the plant; Yapp (81) remarks on the increase of hairs from the centre to the margin of the lamina.

Certain factors appear to influence the number of hairs on some plants

Yapp (81) has observed that with increasing the intensity of sunlight the number of hairs per unit area increases; Vesque and Viet (74) 'accompanied' the variations of sunlight with 'more or less dry air', and obtained the same result. McDougall (55), on the other hand, has found in *Lactuca spicata* that with increasing the sunlight intensity the number of hairs has been diminished. Eberhardt (33) has shown that hairs may be increased in number solely by increasing the dryness of the air surrounding the plant. Grevillius (38) and Stober (69), working independently, note that by increasing the dryness of the soil, exposure to wind and sunlight more hairs are produced; Shreve (65), experimenting with *Encelia farinosa*, also obtained more hairs per unit area in the same species when the aridity of the habitat was increased. A rise in altitude appeared to increase the number of hairs on the same species in Bonnier's (17) experiments.

Solereder (68) enumerates three different structures in clothing hairs, (1) 'simple clothing hairs', (2) 'peltate hairs', including 'stellate and candelabra' forms, and (3) 'shaggy hairs', with which may be classed 'warts and spines'. Not only may each structure occur in many families, but in several families (Capparideae, Ericaceae, Melastomaceae, &c.) the three structures may be found together in one family. Stober (69) has found in general that 'there is a considerable variation in the kind . . . of epidermal hairs not only in different plants of the same species, but also on different leaves of the same plant, or even on different parts of the same leaf'. Consequently clothing-hair structure has been found of no diagnostic value.

The size of the clothing hairs has been found to be variable on one and the same plant and plant-organ in the Angiosperms examined, and this is in accordance with Stober's (69) observations. Zalenski (quoted by Maximov (54)) has observed that the size decreases 'the higher the point of insertion of the leaf and the greater its distance from the root system'.

Thus, it is clear that *clothing hairs* cannot be used in the identification of the vegetative parts of Angiosperms.

### C. *The value of the epidermal cell.*

The characters of the epidermal cell that have been particularly examined for the determination of vegetative organs are the following: (1) The shape of the cell. (2) The size of the cell. (3) The outline or sinuation of the lateral wall of the cell. (4) The thickness of the wall of the cell.

(1) *The shape of the epidermal cell.* In all the Angiosperms examined, cells of the same shape have been found commonly to occur in different families, in different genera of one family, and in different species of one genus.

Cells of different shapes have been commonly found in one and the

same species, plant, or plant-organ; foliage leaves, in particular, have shown cells in the upper epidermis varying considerably in shape from those composing the lower epidermis of the same leaf. These observations are in accordance with Stober's (69) records, and this writer adds that the form of the cell in the lamina is largely determined by the form of the lamina itself. Solereder (68) attributes elongated epidermal cells generally to narrow leaves, 'the long axis' of the cell being 'usually parallel to the median vein'; the narrow leaves of Caryophyllaceae, Papilionaceae, &c., are quoted as exemplifying this.

It is apparent, then, that the *shape of the epidermal cell* is of no diagnostic value.

(2) *The size of the epidermal cell.* The examination of Angiospermous epidermis proved that the size of the epidermal cell may be similar in different families as in Proteaceae and Urticaceae, in different genera of one family as in *Banksia* and *Protea*, and in different species of one genus as in *Ficus benghalensis*, L., and *F. lyrata*, Warb., or in *Myrica californica*, Cham. and Schlecht, and *M. rubra*, Sieb. and Zucc.

Or there may be variation of size in a family as in Lauraceae or Myricaceae, a single genus as in *Ficus* or *Myrica*, a species as in *Acer pseudo-platanus*, L., or *Quercus pedunculata*, Ehrh., or even in one and the same plant (compare the petiole with the lamina of most Angiosperms) or plant-organ (compare the upper with the lower epidermis of the lamina of many Angiosperms). Zalsenski (quoted by Maximov (54)) has noticed on the one plant that the size of the epidermal cell has decreased 'the higher the point of insertion of the leaf and the greater its distance from the root system', and Stober (69) has generally observed on the one plant an increasingly large epidermal cell in passing from the cauline to the radical leaves, and on the one lamina in passing from the apex to the base, and from the lower to the upper epidermis. And in the examination of the Angiosperms enumerated above, the cells composing the upper epidermis of the foliage leaf have been generally larger than those in the lower epidermis of the same leaf; this particular difference has also been noticed in *Fagus sylvatica*, L., by Kny (48). Salisbury (63) and Yapp (81) have found that the size of the epidermal cell on the one plant has been larger in the shade- than in the sun-leaf.

Maximov (54) has grown bean plants in the artificial light of an 'electric lamp of 2,000 candles', placing some plants one metre and others three metres from the light. The epidermal cells were much smaller in the former than in the latter.

Farmer and Chandler (37) remark on the decreased size of the epidermal cells in a plant treated with 'excess carbon dioxide'. Their 'excess' in the air is stated to be 'about 3.5 times the amount of carbon dioxide normally present in ordinary air'.

By increasing the dryness of the surrounding air, Eberhardt (33) obtained a decrease in the size of the epidermal cells on the one plant. Heuser (quoted by Maximov (54)) experimenting with wheat, and Rippel (quoted by Maximov (54)) working with white mustard (*Sinapis alba*, L.) increased the dryness of the soil and obtained a diminution in the size of the epidermal cell on the one plant.

According to Bonnier (17), who grew the same species of plant at different altitudes, the epidermal cell decreased in size with increasing altitude.

These facts prove sufficiently that there is no determinative value in the *size of the epidermal cell*.

(3) *The outline or sinuation of the lateral wall of the epidermal cell.* In the Angiosperms listed above, similarity in the outline of the lateral cell-wall has been commonly found in different families as in Araliaceae and Myricaceae, in different genera of one family as in *Laurus* and *Persea*, and in different species of one genus as in *Aralia Chabrieri*, Hort., and *A. filicifolia*, Chr. Moore, or in *Myrica aethiopica*, L., and *M. californica*, Cham. and Schlecht.

A variation in the outline of the lateral cell-wall also has been commonly found in any one family (Lauraceae, &c.), genus, or species. As instances of generic variation, species of *Cinnamomum* (*C. Camphora*, Nees, &c.) have been found to have cells with straight lateral walls in the lower and upper epidermis of the foliage leaf. Other species of this genus (*Cinnamomum Loureirii*, Nees, &c.) have shown a marked sinuation of the cell-wall in both the lower and upper epidermis of the foliage leaf. And in many species the outline of the lateral cell-wall has varied in one and the same plant; in those cases where the cell-wall of the cells of the foliage leaf has been sinuate (as in *Cinnamomum Loureirii*, Nees, *Persea Linque*, Nees, &c.) the cell-wall of the cells of the petiole or of the stem has generally been straight and in accordance with usual observations, sun-leaves have shown straight-walled cells whereas shade-leaves on the same plant have been characterized by wavy-walled cells. Stober (69), however, has observed cases of sinuosity decreasing from the cauline to the radical leaves, suggesting that with increasing shade the wall has become less wavy. In the case of *Spiraea Ulmaria*, L., Yapp (81) records decreasing sinuosity of the epidermal cell-walls in the progressive stages in the life of the plant, and Zalenski's results (quoted by Maximov (54)) show that the sinuosity of the lateral walls decreases 'the higher the point of insertion of the leaf' on the stem. Rippel (quoted by Maximov (54)) experimenting with white mustard (*Sinapis alba*, L.) found the sinuation of the wall increased with increasing the dampness of the soil. In one and the same plant-organ of the Angiosperms examined, straight lateral walls have usually characterized the upper epidermis of the foliage leaf, whereas wavy

walls have characterized the lower epidermis of the same leaf. But, on the same plant, occasionally, as for instance in *Acer pseudo-platanus*, L., other foliage leaves have been found that have the upper epidermis composed entirely of cells with wavy lateral walls, while straight-walled cells characterize the lower epidermis.

In consequence of this evidence, it is clear that the *outline or sinuation of the lateral wall of the epidermal cell* cannot be used in determinative work.

(4) *The thickness of the wall of the epidermal cell.* Much variation in the thickness of the outer wall of the epidermis has been noticed in one and the same plant in most of the Angiosperms examined.

On the same plant, according to Maximov (54) and Stober (69), the 'cuticle' increases in thickness in passing from the radical to the cauline leaves and Zalenski (quoted by Maximov (54)) has found the thickness increasing 'the higher the point of insertion of the leaf' on the stem. The lamina, also, may show increased cutinization from its base to its apex. In *Spiraea Ulmaria*, L., however, Yapp (81) observed on the one plant that the first leaves were more cuticularized than the later.

Bergen (13), working on the 'Sun Leaves and Shade Leaves of *Olea europaea* and other Broad-leaved Evergreens', has found that the cutinized layer of the upper epidermis has been much more developed in the sun-leaves than in the shade-leaves. Maximov (54) observed the same difference in other sun- and shade-leaves, and Kny (48) obtained the same results in the case of *Fagus sylvatica*, L.

The cuticle appears also to become thicker with increasing the dryness of the air surrounding the plant (Eberhardt (33)), with increasing the dryness of the soil, exposure to wind and sunlight (Stober (69) and Grevillius (38) working independently), or with increased exposure to wind and sunlight alone (Hanson (40)), with increasing the amount of potassium in the soil (Lee and Priestley (49)), or with increasing the altitude (Bonnier (17) and Maximov (54) working independently).

There is evidence, therefore, that the *thickness of the wall of the epidermal cell* is of no diagnostic value.

#### D. *The value of the stoma.*

The vegetative organs of all the genera of Angiosperms mentioned on pages 943 and 944 have been examined for the following characters: (1) The subsidiary cells bordering the stoma. (2) The level of the stoma in the epidermal tissue. (3) The number of stomata per unit area. (4) The arrangement and orientation of stomata. (5) The size of the stoma. (6) The structure of the stoma.

(1) *The subsidiary cells bordering the stoma.* According to Solereder

(68) the arrangement of the subsidiary cells around the stoma conforms in Angiosperms only to three definite types, the number of cells in each of these types varying. From the point of view of diagnosis, it is interesting that each of these types is of widespread occurrence, that numerous families and genera of Angiosperms exhibit each of these types, and that 153 families are listed as having no subsidiary cells at all.

Copeland (26) has noted that subsidiary cells around the stoma often occur in the stem stomata only. This writer also has remarked that in their attachment to the guard-cells the subsidiary cells on the stem may differ from those on the lamina.

These observations have been confirmed in the Angiosperms listed above, and hence all the evidence has shown that the presence or absence of *subsidiary cells bordering the stoma* can be of little systematic value.

(2) *The level of the stoma in the epidermal tissue.* In most of the Angiosperms examined the level of the stoma in the epidermis has been similar in different families as in Malvaceae and Scrophulariaceae, in different genera of one family as in *Campanula* and *Lobelia*, or in *Mentha* and *Nepeta*, and in different species of one genus as in *Lysimachia barystachys*, Klatt, and *L. vulgaris*, L., or in *Armeria boetica*, Boiss., and *A. sardoa*, Spreng. In a few cases (*Acer pseudo-platanus*, L., *Ulmus campestris*, L., &c.) a variation in level on one and the same plant has also been noticeable.

According to Copeland (26) some stomata show a remarkable difference in their level in the epidermis as the guard-cells open and close. Observations prove in many cases that as the pore closes the guard-cells rise.

Therefore, the *level of the stoma in the epidermal tissue* cannot be considered in determinative work.

(3) *The number of stomata per unit area.* In order to find out if an Angiospermous family, genus, or species has a characteristic number of stomata in any special area of any particular vegetative organ many different parts of the plant were examined and similar areas on different plants were compared. In the case of foliage leaves the area investigated and compared on different plants was at the base of the lamina close to the midrib, since stomata were found to be more numerous near the base of the leaf than at the apex, and towards the midrib rather than near the margin of the leaf. This selected area apparently contained, therefore, the maximum number of stomata per unit area in the lamina.

The position of this area accords with Eckerson's (35) results in connexion with many oblong leaves and with observations made by Skene (66), but Salisbury (63) has generally found, and Yapp (81) in *Spiraea Ulmaria*, L., that there is a marked increase of stomata from the base to the apex of the lamina and from the midrib to the leaf-margin.

The figures obtained in the examination of foliage leaves from



comparable regions on the different plants were as follows. (The figure recorded represents the average number obtained for the species):

Family.	Genus.	Species.	Number of stomata in 4.8 sq. mm.
Araliaceae	<i>Aralia</i>	<i>Chabrieri</i> , Hort.	16
	"	<i>filicifolia</i> , Chr. Moore	17
	<i>Pseudopanax</i>	<i>crassifolium</i> , Koch	10
Lauraceae	<i>Cinnamomum</i>	<i>Camphora</i> , Nees	22
		<i>Loureirii</i> , Nees	24
	<i>Laurus</i>	<i>nobilis</i> , L.	10
	<i>Litsaea</i>	<i>ferruginea</i> , Blume	17
	<i>Persea</i>	<i>gratissima</i> , Gaertn.	17
		<i>Linque</i> , Nees	17
		<i>officinale</i> , Nees & Eberm. ( <i>variifolium</i> , Kuntze)	17
Myricaceae	<i>Myrica</i>	<i>aethiopica</i> , L.	22
		<i>californica</i> , Cham. & Schlecht	18
		<i>cordifolia</i> , L.	12
		<i>rubra</i> , Sieb. & Zucc.	25
Urticaceae	<i>Ficus</i>	<i>australis</i> , Willd.	7
		<i>Barteri</i> , Sprague	17
		<i>benghalensis</i> , L.	12
		<i>elastica</i> , Roxb.	6
		<i>insectoria</i> , Roxb.	13
		<i>lyrata</i> , Warb.	25
		<i>nitida</i> , Blume	10
		<i>religiosa</i> , L.	14
		<i>sagittifolia</i> , Warb. <i>Vogelii</i> , Miq.	20 16

It is clear from the similarity of figures in the four families considered that there is no characteristic number of stomata distinguishing one family from another. From the marked similarity of figures in the three genera of Lauraceae (*Litsaea*, *Persea*, and *Sassafras*) and from the wide range of similar figures in varying species of *Ficus* and *Myrica* it is equally clear that there is no particular number of stomata characteristic of any of these genera or species.

Apparently in some Angiosperms there is an increase in the number of stomata per unit area in passing from the base to the apex of the stem (species of *Salicornia*, &c. investigated by Delf (30)), from the stem to the leaf (Copeland (26)), and from the radical to the cauline leaves (Maximov (54), Rea (61), Salisbury (63), Stober (69) and Yapp (81)). Zalenski (quoted by Maximov (54)) and Salisbury (63), working independently, have found stomatal frequency increasing with 'the height of the leaf above the soil'. According to Stober (69), the number of stomata increases with the increasing cutinization of the plant. In the case of *Spiraea Ulmaria*, L., Yapp (81) observes stomatal frequency increasing on the successively opening organs as the life of the plant progresses. Loftfield (53) writes that 'the number . . . of the stomata on any given area of leaf' is 'in-

fluenced by the conditions under which they were formed', and Eckerson (34) has noted that 'marked variations in number . . . of stomata occur, not only in different varieties of the same species, but in the same varieties grown under different external conditions'. Factors such as the intensity of light, humidity of the environment, altitude, the amount of carbon dioxide in the air surrounding the plant are recorded by the following observers as playing important parts in determining this variation of stomatal frequency.

An increasing intensity of sunlight has been stated by Bergen (13), Loftfield (53), Rea (61), Skene (66), Stoll and Willstätter (79), (80) (working together), and Yapp (81) to increase the number of stomata per unit area. Bergen, studying the 'Transpiration of the Sun leaves and Shade Leaves of *Olea europaea* and other Broad-leaved Evergreens', found stomata more numerous on the sun- than on the shade-leaves, 'an average of two determinations gave 15 per cent. excess for the former', and Loftfield (53) has also found that 'a leaf developed in the shade has fewer . . . stomata per unit area than one produced in sunlight'.

Maximov (54) has grown Bean plants in the artificial light of an 'electric lamp of 2,000 candles'. Those plants placed one metre away from the light developed about four times as many stomata per unit area as those grown at a distance of three metres from the light.

According to Loftfield (53), the number of stomata per sq. mm.<sup>1</sup> on a leaf of *Malva rotundifolia* produced in June was 173; another leaf on the same plant produced in 'the hot, dry weather' of the succeeding month had 241 stomata per sq. mm.<sup>1</sup> But the 'ratio of stomata to other epidermal cells was the same, and hence the difference was merely one of expansion or the size of the cells'. Thus a numerical variation was effected by changing the temperature and humidity of the environment. And Grevillius (38) has found that plants grown in a dry habitat with much exposure to wind and sunlight have produced more stomata per unit area than similar species grown under less xerophytic conditions. Salisbury (63) reports on obtaining a higher stomatal frequency by increasing the dryness of the environment. Eberhardt (33) has found that by increasing the dryness of the surrounding air alone, the same result can be effected. Heuser (quoted by Maximov (54)) experimenting with wheat, and Rippel (quoted by Maximov (54)) with white mustard (*Sinapis alba*, L.), have also proved that an increase in the number of stomata per unit area can be obtained by increasing the dryness of the soil, and Kokin (quoted by Maximov (54)) working on other plants produced similar results.

Bonnier (17) notes that the same plant species grown at different altitudes commonly shows an increase in the number of stomata with an increasing altitude.

Farmer and Chandler (37), experimenting on the 'Influence of an

<sup>1</sup> There appears to be some error in the area mentioned, and probably 'sq. mm.' should read 'sq. cm.'. In either case the principle involved is the same.

Excess of Carbon Dioxide in the Air on the Form and Internal Structure of Plants', find that plants grown in air containing about 3.5 times the normal amount of carbon dioxide have more stomata per unit area than those grown in air containing the normal percentage of carbon dioxide (3.29 parts of carbon dioxide in 10,000 volumes of air). In the case of *Solanum atropurpureum* 'the number of stomates per unit area of the leaf surface is greater in the carbon dioxide than in the air plant in the proportion 1.3 : 1.0'. This was found to be the case also in *Kalanchoë welwitschii* and species of *Fuchsia*; in *Begonia gracilis* the proportion was 2.3 : 1.0.

From this collective evidence there is no diagnostic value in the number of stomata per unit area.

(4) *The arrangement and orientation of stomata.* In the Angiosperms examined (see above list), an irregular arrangement and orientation of stomata have been found to be of widespread occurrence in dicotyledonous vegetative leaves usually not elongate in shape. A linear arrangement and roughly parallel orientation have been generally found in elongate dicotyledonous vegetative leaves, in dicotyledonous stems, and in monocotyledonous stems and vegetative leaves. Either arrangement or orientation of stomata has occurred too commonly to be of use in the diagnosis of Angiosperms.

Hence, neither the *arrangement* nor the *orientation of stomata* can be employed for purposes of identification.

(5) *The size of the stoma.* From a study of stomatal movements in the Angiosperms enumerated above, and from the writings and diagrams of Haberlandt (39) and Lloyd (51), it seems fairly general in Angiosperms that, when the stoma opens and closes, the only dimension that remains approximately the same, and consequently may appear to be of diagnostic value, is the whole length of the stoma; for, in accordance with other observers, the length of the stomatal pore alone has been found to vary in the opening and closing of the guard-cells.

But, an extensive examination has shown that the whole length of the stoma may be similar in different families, in different genera of one family, and in different species of one genus. Hence stomatal size (as shown by the length of the stoma) has been found of no use in the determination of Angiosperms.

Nor has it been possible to rely on the average length of the stoma in diagnostic work. The average stomatal length in *Persea gratissima*, Gaertn., and in *Cinnamomum Loureirii*, Nees, has been found to be 0.018 mm., but *Persea gratissima* has a wide range of lengths, from 0.012 mm. to 0.021 mm., whereas *Cinnamomum Loureirii* has a constant length of 0.018 mm. Again, the average length of the stoma in *Ficus Vogelii*, Miq., and in *Ficus lyrata*, Warb., has been found to be 0.017 mm. *Ficus Vogelii*, however, has a range of lengths from 0.012 mm. to 0.025 mm., whereas *Ficus lyrata* has a constant length of 0.017 mm.

Lloyd (51) and Delf (*in lit.*) have observed instability of stomatal length with the opening and closing of the guard-cells. In *Verbena ciliata*, Lloyd (51) reports on an increased stomatal length with the opening of the pore; Delf has observed in some Angiosperms where cutin was present only to a small extent, a diminution of the length of the stoma with the opening of the pore.

Evidently, then, the length or size of the stoma must be regarded as of no importance in the diagnosis of Angiosperms. Solereder (68), however, states that 'especially in extreme cases (very large or very small stomata, occasionally even stomata of two sizes on the same leaf-surface . . .)' the size of the stomata 'may be employed for the diagnosis of species and occasionally even of more extensive taxonomic groups'. But, in the Angiosperms examined, in cases where the stomata are unusually large as in *Pseudopanax crassifolium*, Koch, and species of *Protea*, or unusually small as in *Banksia integrifolia*, L., and *Cinnamomum Loureirii*, Nees, the large and small types of stoma seem to occur over a wide range of families, genera, and species, so that even in these extreme cases the size of the stomata cannot be taken as a diagnostic feature.

In the majority of the genera examined a difference in stomatal size has been commonly found on different organs of the same plant. Stober (69) has noticed a decrease in the size of the stoma on one and the same plant in passing from the radical to the cauline leaves and Zalenski (quoted by Maximov (54)) has reported on the decrease of stomatal size 'the higher the point of insertion of the leaf and the greater its distance from the root system.' Yapp (81), in the case of *Spiraea Ulmaria*, L., has observed a diminution of stomatal size in the successively opening organs with the increasing maturity of the plant.

Certain factors have been said to influence the size of the stoma. Some of these factors appear to be intensity of light, humidity and temperature of the habitat, possibly also the amount of carbon dioxide in the air surrounding the aerial parts of the plant.

Loftfield (53), Salisbury (63), and Skene (66), working independently, note that the stoma is a larger size in the shade than in the sun-leaves of one and the same plant, and Yapp (81), experimenting with *Spiraea Ulmaria*, L., is of the same opinion. But Kny (48), working on the sun- and shade-leaves of *Fagus sylvatica*, L., figures epidermis from both kinds of leaves and remarks on the similarity of stomatal size in both leaves. In order to test these varying opinions, sun- and shade-leaves of many Angiosperms have been studied, and it has been found that the stomatal measurements from both kinds of leaves on the one plant have been very similar.

Salisbury (63) has found in a woodland flora that a plant grown in a moist habitat has larger stomata than the same species grown in a dry

habitat; Heuser (quoted by Maximov (54)), experimenting with wheat, noticed that the size of the stomata increased with increasing the moisture of the soil alone.

Loftfield (53), observing leaves of *Malva rotundifolia* produced 'during the hot, dry weather of the first part of July' and comparing them with those produced a month earlier, remarks on the different size of the stomata in the two sets of leaves. The June leaves had larger stomata than the July leaves.

Farmer and Chandler (37), working on plants surrounded by an excess of carbon dioxide ('about 3.5 times the amount of carbon dioxide normally present in ordinary air') and comparing the form and internal structure of such plants with similar ones grown in air containing a normal percentage of carbon dioxide (3.29 parts of carbon dioxide in 10,000 volumes of air), report on the approximately same size of the 'air' and 'carbon dioxide' plants. If there is any difference between the two plants, the guard-cells of the plant grown in carbon dioxide 'excess' are the greater.

It may be concluded, therefore, that the *size of the stoma* must be regarded as of no diagnostic importance.

(6) *The structure of the stoma.* Detailed research into the value of stomatal structure in the identification of Angiosperms has been done by Solereder (68). This writer states that 'the mode of attachment of the epidermal cells surrounding the stomata to the pairs of guard-cells has proved to be of the greatest systematic importance'. As this is 'intimately connected with the course of development of the stomata from the cells of the dermatogen' this writer has made a close study of the latter; as a result, four modes of development have been determined in Angiosperms and named as follows: Ranunculaceous, Rubiaceous, Caryophyllaceous and Cruciferous.

Recognizing these four lines of development, the writer lists families which have stomata agreeing with one or other of these modes. It is apparent from these lists that most families show more than one course of stomatal development; indeed, Bixineae, Polygaleae, and Vochysiaceae each exhibit three of the above four modes of development. Clearly, then, this method for the identification of Angiosperms is weak.

And further, these four modes of development 'are very commonly quite unrecognizable in the mature leaf', so that (Solereder adds) 'the utilization of the different types of stomata for systematic purposes is involved in great difficulties in practice'. As an instance of the difficulties, the mature stomatal structures of the Ranunculaceous and Cruciferous modes of development are quoted; these are often quite indistinguishable at maturity, though the course of development of the two stomatal modes leading to these final structures has been quite different.

Tognini's investigations, quoted by Solereder (68), prove that 'the

development of the stomata on the various organs (e.g. foliage-leaf, cotyledon, petal, stem) of the same plant-species may either be identical or may vary'.

There seems, therefore, to be no possibility of identifying Angiosperms from a knowledge of stomatal development. Stomatal structure alone remains for criticism.

Solereder (68) has found the following features of the structure 'of great systematic importance':

1. The contour of the pairs of guard-cells.
2. The shape of the front cavity when seen from the surface.
3. The structure of the back cavity.
4. The varied character and chemical nature of the unequal thickening of the walls of the guard-cells.
5. The corresponding differences in the shape of the lumina of the guard-cells.
6. The thickening ridges which arch over the front and back cavities (mostly strongly cuticularized).
7. The epidermal joints found on either side of the guard-cells.

From these facts it is clear that, to obtain a complete picture of the structure of a stoma, a surface view only is most inadequate. It is not surprising, then, that viewed from the surface only, very many different Angiosperms have a similar stomatal structure. And it is not surprising, either, that in fossilized Angiosperms (for instance, the plants from the Pipe-Clay horizon of the Bagshot Beds), where vertical sections have been impossible, stomatal structure has been found of no help in diagnostic work.

But in many Angiosperms stomatal structure viewed from the surface and in vertical section cannot be used for purposes of plant determination. All the plants so far selected from the 153 families enumerated by Solereder (68) as having no subsidiary cells have shown such similarity of structure in surface view and in vertical section that from stomatal structure alone it would be impossible to determine one family, one genus, or one species from another.

And Hryniewiecki (43) (44) has shown in Compositae that species of *Eupatorium*, *Pluchea*, and *Telekia* have stomata so remarkably similar in vertical section that this character of the stoma alone could not distinguish these genera from one another.

Hryniewiecki (43) (44) has also shown by means of figures that dissimilarity of stomatal structure on one and the same plant may also occur in Angiosperms; in *Senecio sarracenicus*, L., *S. kleinioides*, Oliver & Hiern, and in *Dahlia variabilis*, Desf., stomatal structure (as seen in vertical section) on the upper epidermis of the foliage leaf is different from that on the lower epidermis of the same leaf; in a species of *Helichrysum*

two adjacent stomata on a foliage leaf show structural differences in vertical section; in *Platanus occidentalis*, L., young stomata seen in vertical section differ very considerably in structure from mature stomata on the same plant; in *Boykinia rotundifolia*, Parry, and in *S. articulatus*, Sch. Bip., stomata of the foliage leaf are most unlike the stem stomata in vertical section.

Loftfield (53) has noticed that 'the stomata of the upper and lower surfaces of the leaves in most plants are different in their structure'. Sugar-beet and some cereals are said to be 'unique' in having similar stomata 'on both surfaces of the leaf'. This author further states that 'the stomata on the stems when such are present, usually differ materially from those of the leaves in structure and relation to water-supply . . .'; this difference between stem and leaf stomatal structure has occasionally been found by Copeland (26) also. Stober (69) has noticed that the cauline leaves of a plant generally have elongated stomata, whereas the stomata on the stem of the same plant are generally rounder in comparison.

When comparing the stomata of the cotyledons with the stomata of the foliage leaves on one and the same plant a remarkable structural difference has often been found in many of the Angiosperms examined (see above list). This has been observed by others also. Where an elaborate structure has occurred in the foliage leaf, a simple structure has usually characterized the stomata of the cotyledon.

It is clear, then, that the *structure of the stoma* cannot be regarded as of systematic value.

*The Value of Combining the Characters of the EPIDERMIS with the External Characters of FORM and VENATION in the Diagnosis of the Vegetative Parts of Angiosperms.*

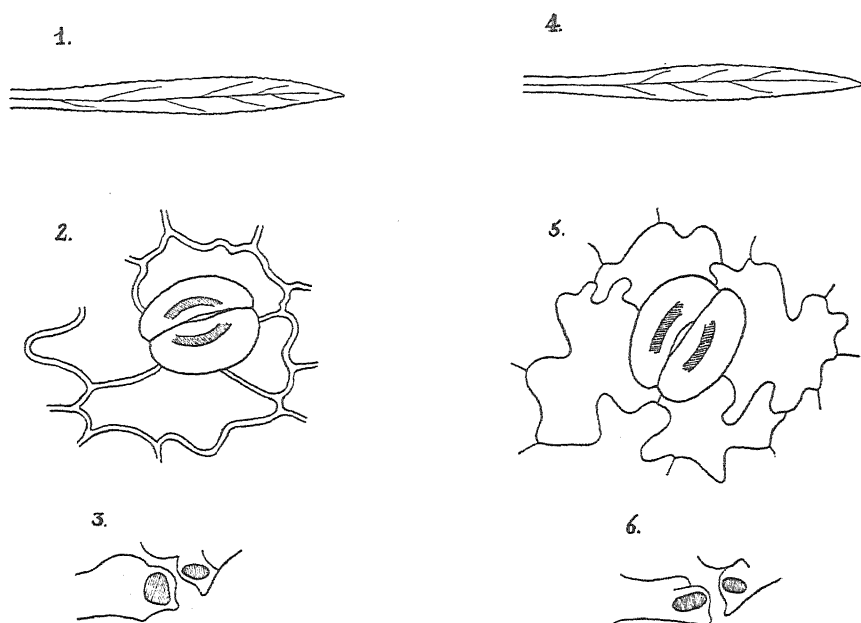
It has been shown in the preceding pages that no feature of the epidermis of the vegetative parts of Angiosperms can be regarded as of diagnostic value.

But, at the present day, in the diagnosis of the vegetative parts of fossil Angiosperms the subsidiary cells bordering the stoma as well as the structure of the stoma are regarded as especially suited (by their peculiarities) to be of specific or even generic value when combined with the external characters of form and venation.

In families where there are no subsidiary cells to the stoma (some 153 noted by Solereder (68)), the leading diagnostic feature of the epidermis must then be stomatal structure, and, in such cases, determinations must be based on a combination of stomatal structure, form, and venation.

It has been easy to find among these 153 families, *stems* of different families agreeing in stomatal structure, form, and venation. Since *foliage*

leaves were found with marked simplicity of form and venation in very many of these families, it was thought possible that foliage leaves agreeing in stomatal structure, form, and venation could also occur in different families.



FIGS. 1-3. 1. *Campanula latiloba*, A. DC. Foliage leaf showing form and venation.  $\times 0.5$ . 2. *Campanula latiloba*. Surface view of stoma on foliage leaf.  $\times 450$ , drawn with the camera lucida. 3. *Campanula latiloba*. Vertical section of stoma on foliage leaf.  $\times 450$ , drawn with the camera lucida.

FIGS. 4-6. 4. *Inula salicina*, L. var. *denticulata* (Borb.) Javorka. Foliage leaf showing form and venation.  $\times 0.5$ . 5. *Inula salicina* var. *denticulata*. Surface view of stoma on foliage leaf.  $\times 450$ , drawn with the camera lucida. 6. *Inula salicina* var. *denticulata*. Vertical section of stoma on foliage leaf.  $\times 450$ , drawn with the camera lucida.

Four only of the 153 families were selected and searched for foliage leaves having the same form and venation. The selected families were Campanulaceae, Compositae, Malvaceae, and Scrophulariaceae. Such leaves were then stripped of their epidermis and the stomatal structure microscopically examined in surface view and in vertical section. The results of this investigation were interesting.

A foliage leaf of *Campanula latiloba*, A. DC. (Campanulaceae), was found to have the same stomatal structure (viewed on the surface and in vertical section), form, and venation as a foliage leaf of *Inula salicina*, L. var. *denticulata* (Borb.) Javorka (Compositae). (Text-figs. 1-6.) A similar agreement was also found between a foliage leaf of *Kitaibelia vitifolia*, Willd. (Malvaceae), and a foliage leaf of *Aster furcatus*, Burgess (Compositae); also between a foliage leaf of *Celsia cretica*, L. (Scrophulariaceae),



and a foliage leaf of *Aster Glehni*, F. Schmidt (Compositae); also between a foliage leaf of *Veronica virginica*, L. var. *japonica* Steud. (Scrophulariaceae), and a foliage leaf of *Actinomeris squarrosa*, Nutt. (Compositae).

Thus, in four cases foliage leaves having the same stomatal structure, form, and venation were found in different families.

#### CONCLUSIONS.

It is shown in this paper that any feature of the epidermis of the vegetative parts of living Angiosperms is unsatisfactory for diagnostic work. It is also shown that by combining the external characters of form and venation with the generally considered strong diagnostic characters of the epidermis (the subsidiary cells and stomatal structure) accurate specific or generic diagnosis of the vegetative organs of living Angiosperms is not ensured.

These results obtained from an examination of *living* Angiosperms doubtless hold good also for *fossil* Angiosperms. It is, therefore, concluded that the modern method of naming fossil Angiosperms from a combination of the form, venation, and epidermal structure of their vegetative organs is quite inadequate for specific or even generic diagnosis. But, however, these three characters combined might, with caution, be utilized if generalized, non-committal names are employed.

This rejection of the *vegetative organs* of fossil Angiosperms as of value for accurate determinative purposes, only serves to emphasize the importance of the *fruits* and *seeds*, for in these organs there is an unquestionably stable determinative value.

#### ACKNOWLEDGEMENTS.

I should like to thank Professor W. T. Gordon for his critical interest and advice throughout the whole period of research.

I feel very grateful, also, to Dr. E. M. Delf, Mr. L. A. Boodle, and Dr. C. R. Metcalfe for their helpful suggestions in matters of technique.

The Director of the Royal Botanic Gardens, Kew, has most kindly supplied me with many of the modern plants mentioned in this paper and has given me unique facilities in the Gardens and Jodrell Laboratory for studying the vegetative organs of a great number of Angiosperms.

The fossil plants from the Pipe-Clay horizon of the Bagshot Beds were collected from the South Western Potteries, Parkstone, Dorset, and from the Norden (Arfleet) Clay Works, Corfe Castle, Dorset. To the owners of these respective clay-pits I tender my grateful thanks for permission to visit their works and to collect there. I am also indebted to the Director of the Kinson Pottery, Constitution Hill, Parkstone, Dorset, for allowing me access to his clay-pits.

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# Chromosomes of *Taxus*, *Sequoia*, *Cryptomeria* and *Thuja*.

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With twelve Figures and two Diagrams in the Text.

THE great majority of the species of Gymnosperms (excluding the peculiar group Gnetales) in which chromosome counts have been made, possess the haploid number of 12. There are, however, a number of records of aberrant numbers, some of the observations dating from the early days of cytology, and many of them incidental to morphological studies. The present work consists in a re-examination of some of the species in which aberrant numbers have been reported, together with a fuller account of chromosome behaviour in *Taxus*, which provides in its pollen mother-cells very suitable material for the study of meiosis, and particularly of the prophase stages.

## MATERIALS AND METHODS.

Counts of chromosomes in the following species and varieties were made:

	Root tips.	P.M.C.
<i>Taxus baccata</i> , L. . . . .	24	12 <sup>II</sup>
„ „ <i>fastigiata</i> . . . . .	—	12 <sup>II</sup>
„ „ <i>adpressa aurea</i> . . . . .	—	12 <sup>II</sup>
„ <i>cuspidata</i> , Sieb. et Zucc. . . . .	24	—
„ „ <i>contorta</i> . . . . .	24	—
„ <i>canadensis</i> , Willd. var. <i>aurea</i> . . . . .	—	12 <sup>II</sup> + I
<i>Sequoia sempervirens</i> , Endl. . . . .	ca. 50	—
<i>Cryptomeria japonica</i> , D. Don. . . . .	24	—
<i>Thuja occidentalis</i> , L. . . . .	24	—

Root tips, obtained from cuttings that had been growing in small pots for several months, were fixed in 2 BE (La Cour, 8) and sectioned at 24  $\mu$ .

Male cones were collected in November. When sample anthers

examined in aceto-carmin showed divisions, the remainder of the cone was smeared. Medium Flemming and Benda were found to give the best fixations.

For all staining, iodine gentian violet was used. (For details of technique, see La Cour, 8.)

The *T. baccata* observations were made from slides prepared by the late W. C. F. Newton.

### *Somatic Chromosomes.*

Root-tip divisions in the Gymnosperms examined could be interpreted only in a few very favourable cases, owing to the long thin chromosomes being crowded at metaphase upon a very narrow equatorial plate.

In *T. baccata*, no evidence could be found of either sex being heterogametic, for in both there are two chromosomes with subterminal attachment constrictions and twenty-two with submedian (Figs. 1 and 2). Study of the chromosome morphology cannot be carried further, since drawings can give practically no idea of the appearance of the complement, owing to most of the length of the chromosomes lying perpendicular to the equatorial plate.

Because so many chromosomes were present in the narrow cells of *Sequoia sempervirens* it was not possible to disentangle any division accurately, and all that could be done was to determine that the number was near to fifty.

The chromosomes of *Cryptomeria japonica* (Fig. 3) were thicker and shorter than those seen in the other genera. This difference in chromosome size may be considered to be genetically controlled (see Darlington, 2).

### *Meiosis in T. baccata.*

The early prophase stages appeared to follow the course for a normal diploid plant with an unpolarized zygotene, as described by Darlington (1). Fig. 4 shows portions of a nucleus at early diplotene—the bivalents being too tangled and numerous to allow of the entire nucleus being followed out as has been done in *Anemone* (Moffett, 11) and *Bellevalia* (Dark, see Darlington, 3). The typical diplotene loops between chiasmata are just beginning to form. Each arm of a loop can be seen distinctly to be double and the thread between loops appears to be double. There are two possible explanations of the double appearance between loops.

(1) The loops mark the places where the chromosomes that paired at zygotene have divided longitudinally into chromatids; so that the ends of the loop indicate the points where division was actually occurring when fixation of the nucleus took place.

(2) The chromosomes between the loops may be actually divided, but

the quadruple nature of the thread is undetectable owing to insufficient optical resolving power. From this it would follow that the characteristic

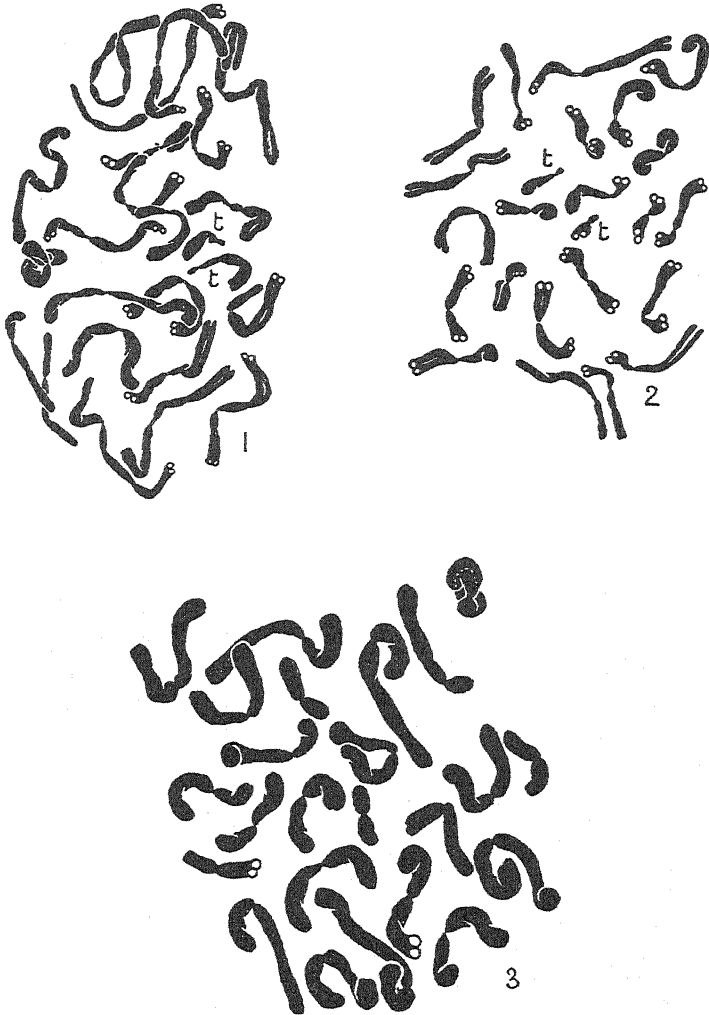


FIG. 1. Metaphase of mitosis in root-tip division from a male plant of *Taxus baccata*. The chromosomes with subterminal attachment constriction are marked *t*.  $\times 3,000$ .

FIG. 2. The same from a female plant.  $\times 3,000$ .

FIG. 3. Mitotic metaphase from root-tip of a male *Cryptomeria japonica* plant.  $\times 3,000$ .

repulsion between pairs of paired chromatids is not present when they are first formed. Hence the first explanation is the more likely one.

At middle diplotene the homologous chromosomes can be seen associated in pairs by one to three chiasmata per bivalent (Fig. 5).

The bivalents at early diakinesis are still as long as they were at



middle diplotene, but they are two or three times as thick and they stain very much more heavily (Fig. 6). This increase in volume is similar to

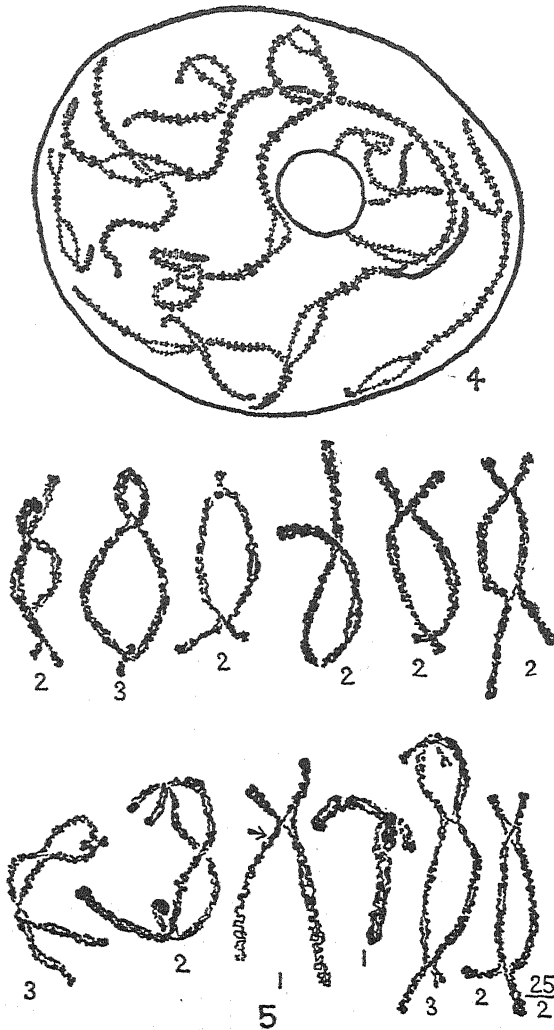


FIG. 4. Part of a pollen mother-cell at early diplotene in *T. baccata*.  $\times 3,000$ .

FIG. 5. A completely analysed nucleus at middle diplotene in *T. baccata*. The number of chiasmata in each configuration is given, together with the total number of chiasmata and number of terminal chiasmata for the nucleus.  $\times 3,000$ .

that found in *Stenobothrus*. By the commencement of metaphase (Fig. 7) the bivalents have condensed to about half their diplotene length, and as metaphase advances (Fig. 8) they assume a smoother outline and become spread out evenly on the equatorial plate of the spindle.

At anaphase (Fig. 9) the chromatids come apart from the chiasmata,

which have so far held the homologous chromosomes together as bivalents, allowing the homologous chromosomes to pass to opposite poles of the cell to form daughter nuclei. After an interphase period the usual second division takes place to produce tetrads.

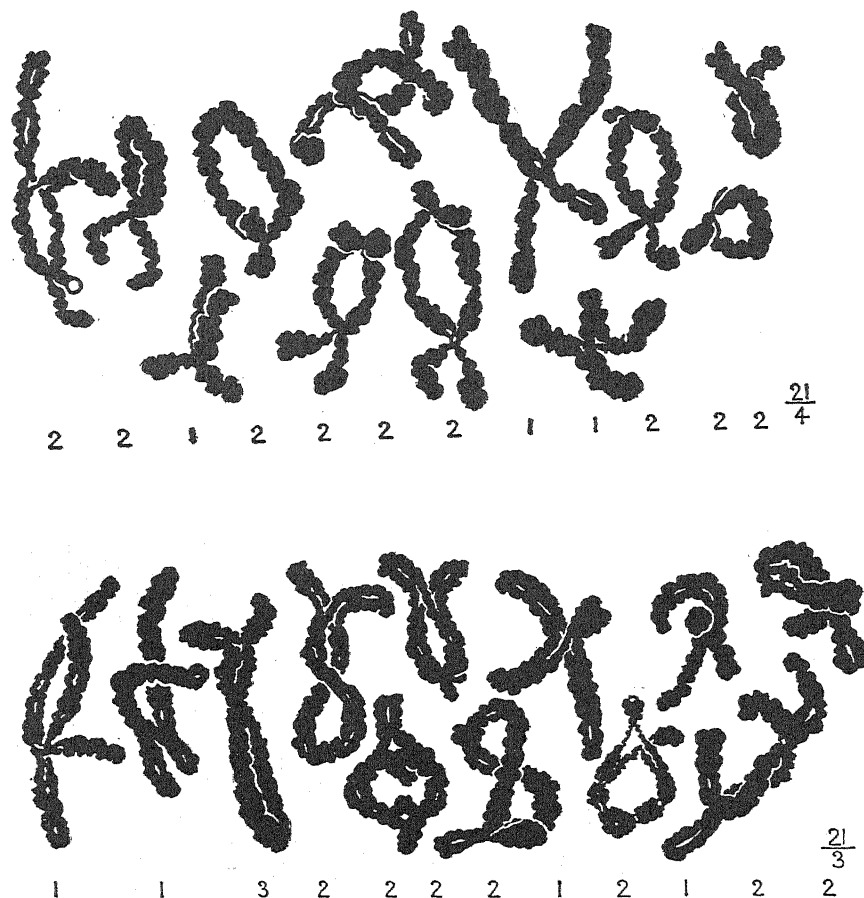


FIG. 6. The chromosome complements of two *T. baccata* pollen mother-cells at diakinesis. Above,  $\times 3,300$ . Below,  $\times 3,000$ .

In order to measure the variation in size that had been noticed in the chromosomes at various stages of meiosis, a comparison of the sizes of the nuclei at these stages was made (Table I). A measure of the volume of the nucleus was obtained by taking the cube of the mean radius of a camera lucida drawing of the outline of the nucleus. As there is no nuclear membrane at metaphase, the nucleus was, for purposes of comparison, arbitrarily represented by a sphere constructed with the equatorial plate as the diameter. This gives a rather high value as indicated by

comparison with the pro-metaphase determination (a stage at which the chromosomes have the typical metaphase bulk but are still distributed in a spherical space).

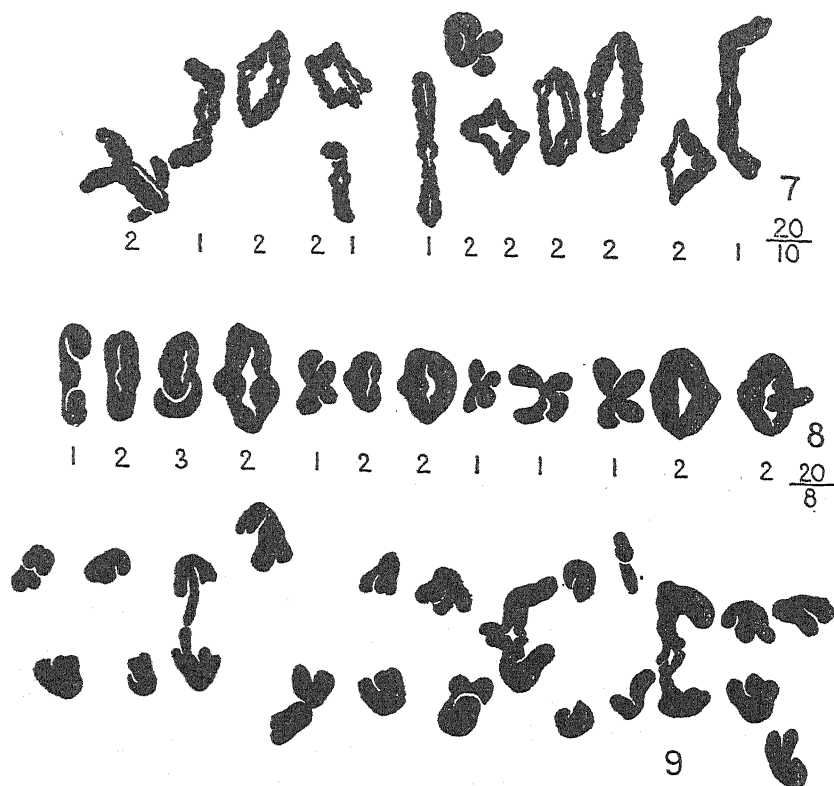


FIG. 7. Side view of early metaphase in pollen mother-cell division of *T. baccata*.  
× 3,000.

FIG. 8. Side view of full metaphase in *T. baccata*. × 3,000.

FIG. 9. Side view of anaphase in *T. baccata*, showing separation of the paired chromosomes by pulling apart of chromatids from chiasmata.

TABLE I.  
*Taxus baccata*.

Stage of nucleus.	Comparative volume.
Pachytene . . .	53
Diplotene . . .	65
Diakinesis . . .	100
Pro-metaphase . .	13
Polar metaphase . .	17

These differences in size are probably related to variations in the condition of the colloidal system of the nucleus, which cause it to absorb water until

a maximum bulk is reached at diakinesis, after which a rapid reversal of the process occurs during the progress of metaphase. A similar variation in bulk with the stage of division is found in *Stenobothrus*, while in other

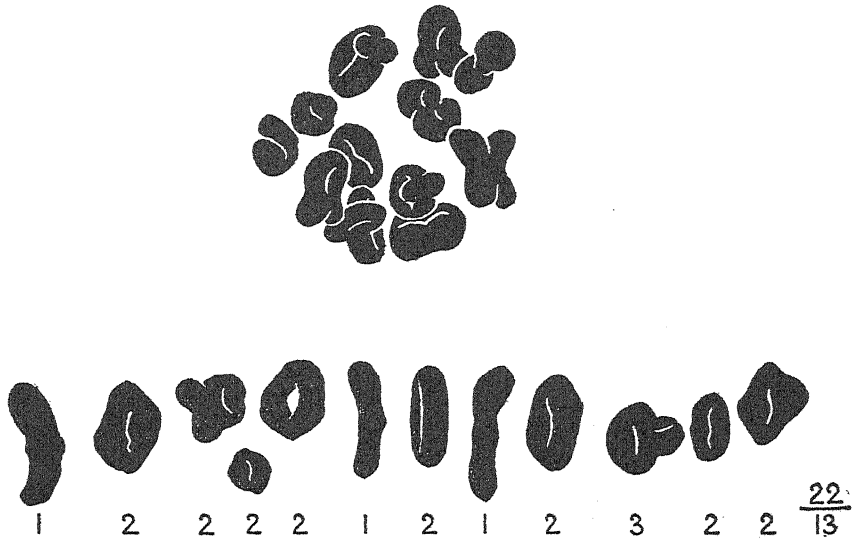


FIG. 10. Metaphase of pollen mother-cell divisions in *T. baccata fastigiata*. Above, polar view. Below, side view.  $\times 3,000$ .

organisms, such as *Tulipa* and *Anemone*, the chromosome bulk remains approximately constant throughout meiosis.

In material of the varieties *T. baccata fastigiata* (Fig. 10) and *T. b. adpressa aurea* (Fig. 11) stages earlier than metaphase were not found. The chiasma frequency at this stage may be a little higher than in *T. baccata* (see Figs. 8, 10, 11), but the evidence is inconclusive, as interpretation of the very condensed metaphase configurations must be checked by comparison with the figures obtained at earlier stages of meiosis.

*T. canadensis*, however, differs from the other forms examined in that the chromosome complement at meiosis consists of twelve bivalents plus a small chromosome assumed to have arisen as a fragment. Only metaphases have been examined, and all attempts at obtaining root-tips have so far failed. Hence one cannot yet tell whether this condition has arisen newly in the sexual cells of this individual tree, or is an old-established somatic state in the species. The fragment, as in analogous cases in other organisms, is quite free in most of the divisions examined. In one or two cases there is a possibility of its being paired to a bivalent, but they are not sufficiently clear to be conclusive. Fig. 12 shows polar and side views of metaphase plates with the free fragment. The division shown at the bottom in Fig. 12 is at present inexplicable. The configuration X can be

explained on the basis that a fragment is homologous with parts of the members of a bivalent and has paired with them as in the diagram. The problem remains, however, of why there should be two fragments in the nucleus.

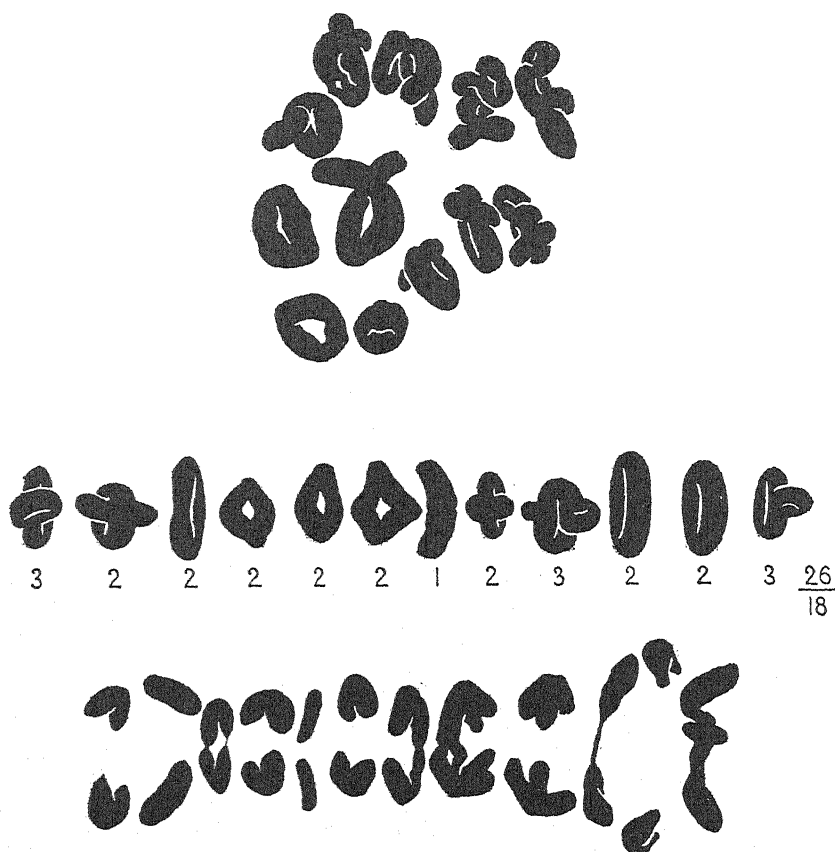


FIG. 11. Divisions in pollen mother-cells of *T. baccata adpressa aurea*. Above, polar and side views of metaphase. Below, anaphase.  $\times 3,000$ .

#### *Consideration of the Chiasmata of T. baccata.*

Counts were made of the chiasmata in whole nuclei at the stages of diplotene, diakinesis, and metaphase. The frequency polygons of chiasmata per bivalent at each stage were plotted (Diagram 2). These show that there has been a reduction in the number of chiasmata in passing from diplotene to diakinesis, while the latter stage is substantially the same as at metaphase.

There is a slight discrepancy between the diakinesis and metaphase figures. As prophase and metaphase divisions were not found in the same

preparation, it was thought that the conditions governing chiasma formation in the two sets of observed configurations might have been altered by external influences. Such a factor has been inferred to be acting in *Aegilops-Triticum* hybrids (Kihara, 7) and in *Vicia Faba* (Maeda, 10) by

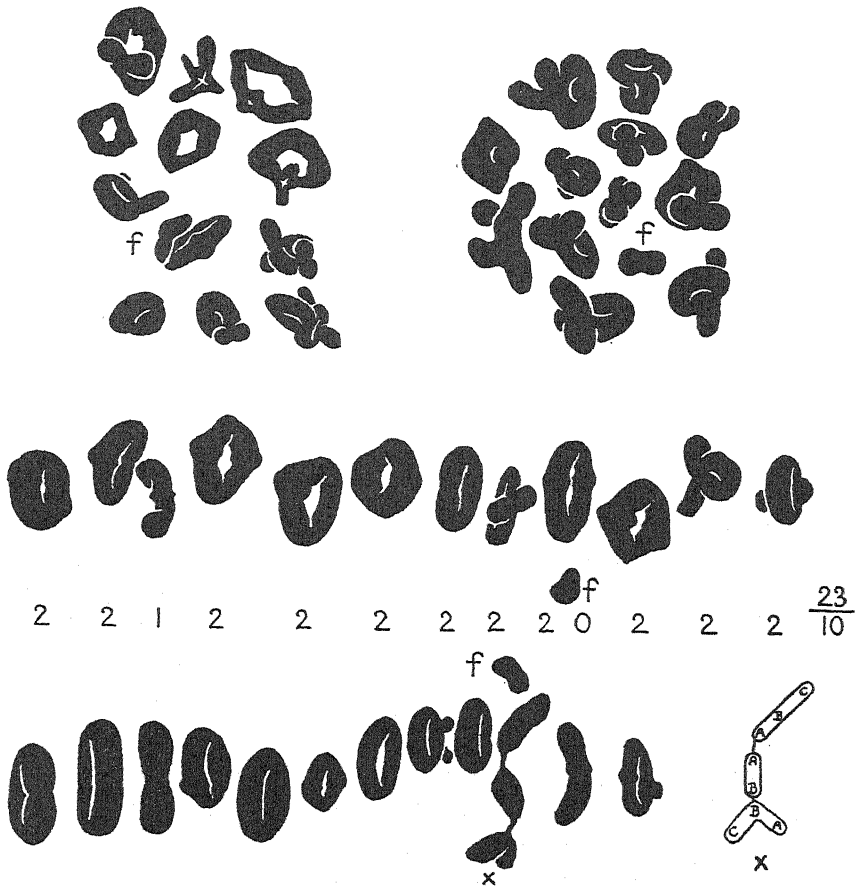


FIG. 12. Metaphase in pollen mother-cells of *T. canadensis*. Above, two polar views of metaphase. Below, two side views of same. *f* indicates the small extra chromosome. The diagram is an explanation of the configuration  $\times \times 3,000$ .

Darlington (1). Statistical data showed that probably there is no such factor acting, for the value of  $X^2$  for the diakinesis and metaphase distributions is 4.82, which from Fisher's tables gives a value of  $P$  lying between 0.05 and 0.10—indicating that there is no significant difference between the chiasma distributions at these two stages.

In Table II all the frequency classes at each stage of meiosis are combined.

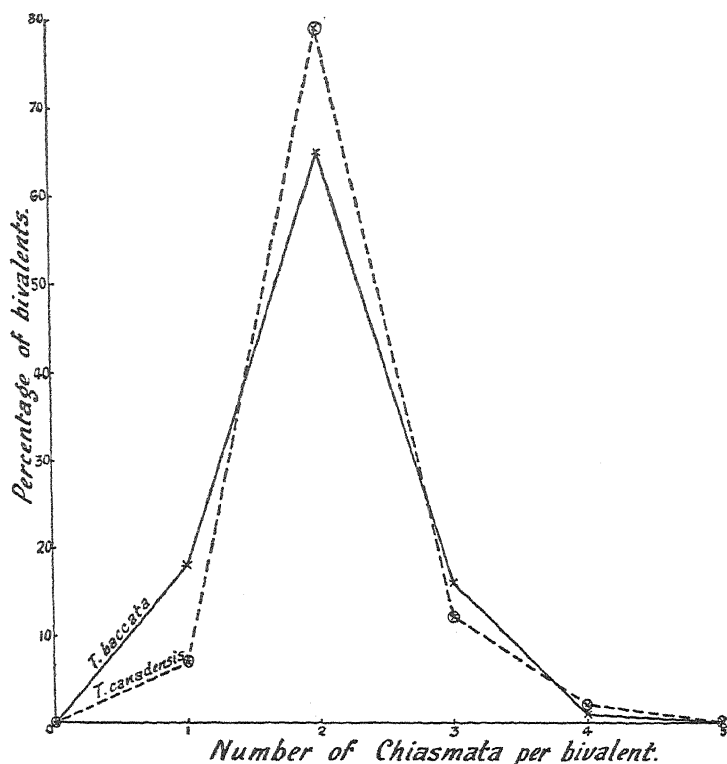


DIAGRAM I.

TABLE II.

*Taxus baccata*.

Stage.	No. of nuclei.	Chiasmata.	Terminal chiasmata.	Number of	
				$X^{ta}/biv.$	Term $X^{ta}/biv.$
Diplotene	4	105	8	2.2	0.17
Diakinesis	8	162	20	1.7	0.21
Metaphase	12	245	108	1.7	0.75

Terminalization coefficient (at metaphase) 0.44.

From this table it can be seen :

(1) There is a slight reduction in the number of chiasmata from diplotene to diakinesis, after which stage no more are lost.

(2) Terminalization (the movement of chiasmata to the chromosome ends) occurs in two stages, (a) during the transition from diplotene to diakinesis there is a little terminalization resulting in the loss of some chiasmata. This terminalization probably occurs chiefly in bivalents having more than two chiasmata, and forces the interstitial ones into one another at the chromosome ends, thus reducing the number of chiasmata.

Between diakinesis and metaphase a vigorous terminalization occurs, so that at the latter stage nearly half the chiasmata are terminal. The terminalization coefficient (number of terminal chiasmata divided by total

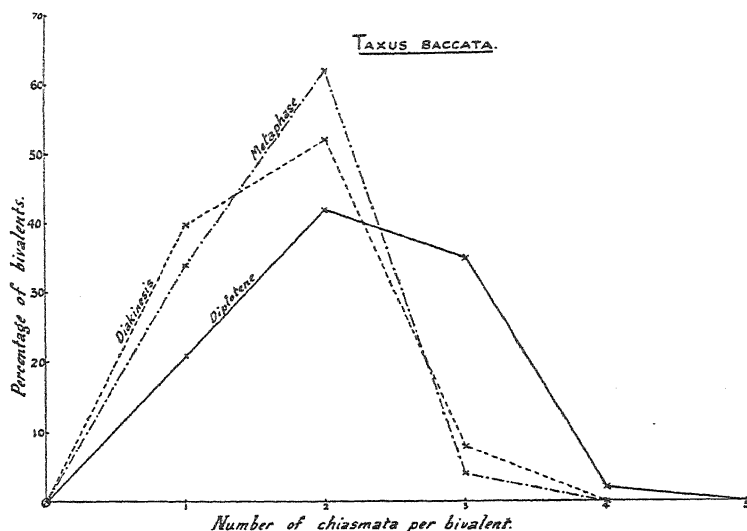


DIAGRAM 2.

number of chiasmata) has not been employed, as it is not helpful in the present investigation.

To compare the chiasma distributions in the two species of *Taxus* studied, the frequency distributions in all available divisions from the stage of diakinesis onwards were combined to give the polygons in Diagram 1 (*T. baccata* includes the varieties that were examined). The two figures can be seen to be similar, and the curves both show by their narrow form that genetical interference is present (see Haldane, 5), this being more marked in *T. canadensis* as would be expected—it being a more sharply defined species.

#### DISCUSSION.

The results reported in this work show that chromosome behaviour in the Gymnosperms is similar to that described in many Angiosperms and Orthoptera. The average chiasma frequency is low and the presence of interference is indicated. Very regular reduction divisions are attained through the chiasma system that has been developed, in which regular pairing at prophase is ensured by a moderately high frequency of chiasma formation between the members of a bivalent, followed by a reduction of the number through terminalization, which simplifies the anaphase



separation and results in regular disjunction and the absence of lagging chromosomes.

In preparations of the pollen mother-cells of *T. baccata* very few of the side views of metaphase nuclei show all twelve bivalents, owing to their being superposed in the plane of vision. This may have been the reason why previous counts of the chromosome number in this species were incorrect. In polar views of metaphase divisions, however, twelve bivalents can generally be seen quite plainly.

Some novel features in the pollen mother-cell divisions are reported by Hawker (6), but from the illustrations it would appear that they are due to interpretation of incomplete nuclei. For example Fig. 5, stated to show metaphase 'tetrads', is more likely to be an incomplete anaphase, the chromosomes being characteristically arranged in two groups. Similarly Fig. 6, showing the chromosomes in two planes, could not represent a metaphase plate as stated, but is probably an incomplete anaphase like Fig. 10. The remainder of the illustrations do not show the number of chromosomes. No indication is given of the thickness at which the sections were cut.

The hypothesis that twelve is the basic haploid number in the Gymnosperms, apart from the Gnetales, has been put forward by Tischler (13) and it would seem that further investigation will establish its truth. Such a uniformity is even more remarkable than that obtaining in the Acrididae (Wilson, 15). At present there are fifteen species, other than those of *Taxus*, in which a number other than twelve has been found. Of these, fourteen are tentative counts—in several cases stated by the authors to be merely estimates—incidental to morphological studies, and almost certainly made in cut nuclei. The fifteenth and remaining case is that of *Cycas revoluta*, in which Nakamura (12) finds that though the diploid number is twenty-four, the pollen mother-cells contain only eleven bivalents or occasionally ten bivalents and two univalents. The interpretation is evidently open to criticism, as sections cut at  $12\mu$  were employed; at this thickness the cells would almost certainly be cut and a small bivalent could easily be lost.

Lawson's (9) account of *Sequoia sempervirens* is of interest, for although he could only estimate the haploid number (in nucellar divisions) he gave it as sixteen. This suggests the possibility of polyploidy. Goodspeed and Crane (4) could not determine the number in *S. sempervirens*, but found *S. gigantea* to have twenty-four somatic chromosomes. My own observations show that *S. sempervirens* has about fifty chromosomes in somatic divisions. Hence this species appears to be a tetraploid form—a condition possibly connected with the remarkable powers of regeneration shown by the stumps of felled trees (Veitch, 14).

## SUMMARY.

1. Counts of the chromosome numbers in various species and varieties of *Taxus* showed the basic number to be twelve.

2. *Taxus canadensis* was found in meiotic divisions to have twelve bivalents plus a small, generally unattached, chromosome.

3. *Sequoia sempervirens* is considered to be a tetraploid species.

4. Meiosis in the Gymnosperms examined follows a course similar to that described in many Angiosperms and Orthoptera. The chiasma frequency at metaphase is low (about two) and terminalization is not complete.

I am greatly indebted to Dr. Darlington for his help and criticism throughout this work, and to the Director of the Royal Botanic Gardens, Kew, for permission to obtain material.

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# Microsporogenesis in *Phoradendron*.

BY

FREDERICK H. BILLINGS.

With twenty-four Figures in the Text.

ACCORDING to Jepson (5) there are five species of the heterophytic genus *Phoradendron* within the boundaries of California, several being found in Southern California in the vicinity of Redlands. The most common form is *P. flavescens*, Nutt. var. *macrophyllum*, Engelm., which is parasitic chiefly on the cottonwood, *Populus fremonti*, Wats. In subsequent references to this species the variety name will be omitted for the sake of brevity. A second species is *P. villosum*, Nutt., which is parasitic on the live oak, *Quercus agrifolia*, Neé. A third species, described under the name of *P. californicum*, is found on the honey mesquite, *Prosopis juliflora*, DC., in the vicinity of Whitewater on the edge of the Colorado desert. A form closely related to *P. flavescens*, Nutt. var. *macrophyllum*, Engelm., is the species *P. flavescens* (Pursh), Nutt., which has not been reported as far west as California, but according to York (16), is found in Texas, with a range well to the eastern part of the United States. Of the three species mentioned as occurring in the vicinity of Redlands, *P. flavescens* and *P. villosum* have been the chief basis of study in connexion with the preparation of this paper for the reason that they have been the most easily accessible and have afforded the greatest number of stages in development.

A fortunate circumstance, perhaps, has been the close association of staminate and carpellate plants, both of which may occur not only on the same tree but even on the same branch, their pendant shoots freely intermingling. Thus the conditions have been most favourable for pollination and resultant amphimixis, with stages in endosperm and embryo development, a report on which is the expectation of the author for the near future. York (17) calls attention to the difficulty of finding staminate plants of the Texas form, *P. flavescens* (Pursh) Nutt., and states that berries are nevertheless produced in abundance on the carpellate plants, even though they are remotely distant from any source of pollen. Thoday and Johnson (12) report that in *Arceuthobium pusillum*, which is heterophytic, the two types of plants are seldom found together, even on the same tree.

The agent of pollination in the species under investigation is probably wind. Insects were not seen visiting the flowers, which are inconspicuous and seemingly without odour. Pisek (7) claims that wind is the chief agent in *Viscum album*, and bases his findings on the development of pollen-tubes and embryo-bearing fruits on plants which had been covered with a cloth. Pollen-tubes have been observed in the styles in *Phoradendron* accompanied by a true fertilization.

The blossoming period of *P. flavescens* (Pursh), Nutt., in the eastern part of the United States is given by Britton and Brown (2) as May to July. That of the California species, *P. flavescens* and *P. villosum*, extends over a longer period. The first collection of material of *P. flavescens* was made late in November, 1930, at which time the staminate flowers were in all stages of development with the oldest shedding pollen. The pistillate flowers also showed various stages except that the oldest were not fully open. With the floral buds were large quantities of mature berries. The first collection of *P. villosum* was made in December, and the same stages were found as in the case of *P. flavescens*. *P. californicum* was not collected until February 1931, when both staminate and carpellate plants were in full bloom. Ripe berries were in abundance. The time had passed for the earliest stages in floral development. Subsequent visits to Whitewater, where *P. californicum* is found, showed only developing fruits, and, for a time, ripe berries. During the collection of material of *P. flavescens* in February it was noticed that the plants were in full bloom. Young floral branches in all stages of development gave promise of a continuation of the blossoming period for some time to come. This species was at no time without floral buds, though in the autumn process of development slowed down. The last observation, made early in November, showed staminate buds, with open flowers shedding pollen. Pistillate buds, though far developed, were still closed. *P. villosum* exhibited in general a similar blossoming history. In September both plants were found to be in full bloom. The desert species, *P. californicum*, showed one flowering period only, in early spring. The other two species were in a process of floral bud formation and blossoming during a large portion of the year.

Floral branches in the leaf axils generally occur in groups of two or three, but occasionally of four or five. Each branch consists of a series of segments of two sorts, one studded closely with flowers sunken in the rachis, the other sterile, alternating. The number of fertile segments varies from two to as many as ten as an extreme, a ten-segment branch being about 10 cm. in length. An average length is 5 cm. The flowers have a perianth of three, sometimes four lobes (Fig. 1). Each pistillate flower has small rudiments which correspond to the stamens, while the staminate flower possesses a central column of tissue that probably

represents a rudimentary style (Fig. 2). In the pistillate flower there is a sclerenchymatous tissue underlying the placenta upon which the much reduced ovules are developed; but there is no sign of such a tissue in the

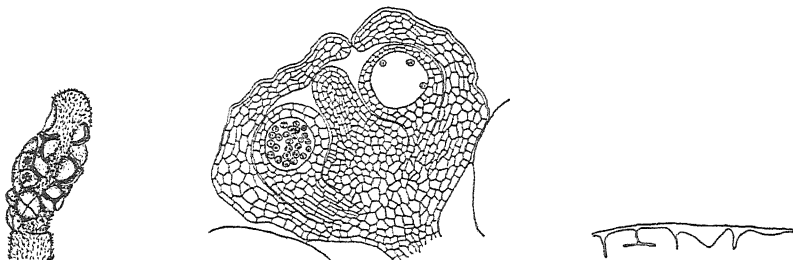


FIG. 1.

FIG. 2.

FIG. 3.

FIG. 1. Terminal segment of a fertile branch of *P. flavescens* var. *macrophyllum* showing staminate flowers.  $\times 5$ .

FIG. 2. *P. flavescens* var. *macrophyllum*. Longitudinal section of a staminate bud showing stamens and rudimentary gynoeceum.  $\times 35$ .

FIG. 3. *P. villosum*. Staminate flower primordia.  $\times 40$ .

staminate flower, nor is there any vestige of a placenta or cavity that would mark the place where ovular development in the pistillate flower occurs.

All material was fixed in Carnoy's fluid, penetration being hastened by partially exhausting the air with a hand pump. When the material was in 95 per cent. alcohol a high-vacuum pump was used to facilitate the entrance later of the embedding substance. The floral branches were mounted in glycerine jelly in close rows upon square pieces of cardboard. After dehydration the specimens thus mounted were embedded in nitro-cellulose, according to the method developed by Professor E. C. Jeffrey of Harvard University. Sections were cut with a Thompson-Jeffrey sliding microtome, and stained in Heidenhain's iron-alum haematoxylin. Drawings were made with the aid of a camera lucida.

A survey of the literature of the Loranthaceae indicates that the genera which have been chiefly studied are *Viscum*, *Arceuthobium*, *Loranthus*, and *Dendrophthora*. Investigation of anther development and meiosis has been principally confined to the labours of Pisek (8, 9). Thoday and Johnson (13) have reported on the flowers and fruit of *A. pusillum*, Peck. Apparently nothing has been done on *Phoradendron*, especially in the fields of microsporogenesis and embryo-sac development.

The staminate flower of *P. villosum* begins as a mere papilla embedded in the floral axis (Fig. 3). The perianth arises as a marginal elevation, leaving a flat, central region which becomes enclosed by the perianth segments. Near the inner attachments of the segments the stamen primordia take their origin. Later, in the centre, a column of tissue arises that simulates, and is probably the morphological equivalent of a style (Fig. 2).

The stamens have very short filaments, so that the ripe anthers appear to be sessile.

The first step in the development of an archesporial tissue is an elongation of a few hypodermal cells in a young anther (Fig. 4), followed

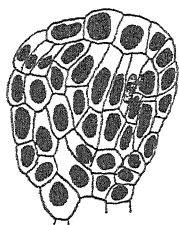


FIG. 4.

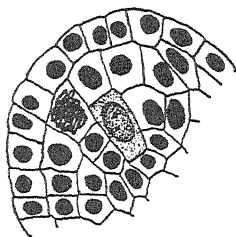


FIG. 5.

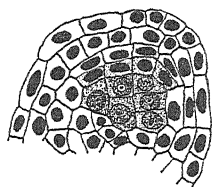


FIG. 6.

FIG. 4. *P. villosum*. Longitudinal section of a young anther showing elongation of hypodermal cells previous to division by periclinal walls.  $\times 200$ .

FIG. 5. *P. villosum*. Primary sporogenous cell.  $\times 200$ .

FIG. 6. *P. villosum*. Origin of tapetum (stippled cells with black nuclei) from inner parietal layer. Several-celled sporogenous tissue.  $\times 150$ .

by division by means of periclinal walls. One of the inner cells thus cut off becomes the primary sporogenous cell. This cell enlarges and is soon sharply distinguishable from the surrounding tissue, not only by its greater size but by its large nucleus and dense cytoplasm (Fig. 5). It undergoes division, and gives rise to a group of sporogenous cells that come to lie near the centre of the anther, because of increase in the number of cell layers between them and the epidermis. These layers take their origin in the hypodermal row which by periclinal walls forms an outer subepidermal row that develops into the endothecium, and an inner layer which is adjacent to the sporogenous tissue. It is from this latter layer, by the formation of periclinal walls, that the tapetum originates as a well-developed jacket of uninucleate cells with rich protoplasmic contents (Fig. 6).

Nuclei are relatively large in all young cells of *Phoradendron*, also of other genera of Loranthaceae in so far as they have been figured. Pisek (9) gives 22–26  $\mu$  as the diameter of the nuclei in the microspore mother-cells of *V. album* at synapsis, and those of *A. oxycedri* as 12–14  $\mu$ . Resting nuclei in the mother-cells of *P. flavescens* measure about 14  $\mu$  in diameter if circular in outline.

A cross-section through a young staminate flower in the microspore mother-cell stage is shown in Fig. 24. These are two archesporia only in each anther, each of which is initiated as a single archesporial tissue with its tapetum. A similar anther structure for *Loranthus europaeus* is claimed by Pisek (8). This author reports the absence of a tapetum in *V. album*. Thoday and Johnson (12) found a tapetal cell layer present in *A. pusillum*. *A. oxycedri* is said to possess a periplasmodium (Schnarf (10)).

From material gathered in late November and December the first study was made of anther development and meiosis. Such study proved of special interest because practically nothing had been done in this field on *Phoradendron*.

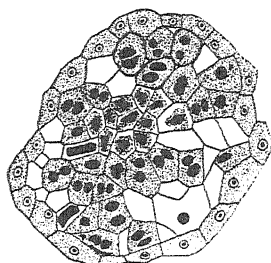


FIG. 7.

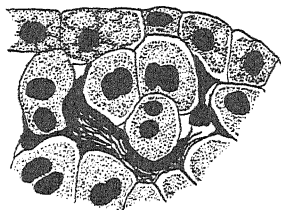


FIG. 8.

FIG. 7. *P. flavescens* var. *macrophyllum*. Section showing interior of anther. Destruction of microspore mother-cells, and their apparent use as tapetal cells. The surrounding layer is tapetum. Four binucleate cells near the tapetum are thickening the exine.  $\times 150$ .

FIG. 8. *P. flavescens* var. *macrophyllum*. Crushing of microspore mother-cells by maturing pollen-grains. Surrounding cells are tapetum.  $\times 300$ .

One of the first discoveries made was that heterotypic divisions in the microspore mother-cells were rarely simultaneous in the same anther, even though it was possible to examine a large number of flowers by the nitro-cellulose method. They occurred singly and seemingly infrequently, although difficulty in finding them may have been due to collection of material at an unfavourable time in the twenty-four hour period. It was also noticed that after the homotypic division the microspores were not arranged in the regular tetrads characteristic of most plants, although all stages were available to the binucleate pollen-grains. The cross-section of an anther in which the binucleate grains occurred appeared as a solid tissue with degenerating and empty cells interspersed through it. That the pollen-grains mentioned were not diads was evident from the thickening of the cell-walls in a manner characteristic of maturing normal grains (Fig. 7). Investigation of the degenerating cells among the microspores showed them to be spore-mother-cells whose dissolution was probably in the functional capacity of tapetal cells, that is, for the better nourishment of the maturing pollen. Each cell thus functioning shows at first a thinning of the cytoplasm, the nucleus then becoming irregular or ragged in outline. Complete crushing of the cell from the pressure of the surrounding tissue finally results (Fig. 8). The employment of microspores in the capacity of tapetal cells is probably of uncommon occurrence, but this is not the first instance that has been recorded. Caldwell (3) in 1899 brought to light a similar condition in *Lemna minor*.

As was stated above, there was no definite arrangement of developing



microspores into the usual diads and tetrads, the mother-cells merely enlarging, their nuclei undergoing division which is unaccompanied by wall-formation. That this division is heterotypic is indicated by the chromosome count. Ten chromosomes pass to one pole and eleven to the other, the somatic number in the staminate plant being twenty-one. The homotypic division was not recognized as such in this winter material.

At the time these observations were made it was thought that microsporogenesis proceeded in the manner briefly outlined above, and that the irregularities noted were related to the parasitic mode of life. It was not until material collected in the spring and summer months showed a somewhat altered course of events that it was realized that time of year exerted an influence. Material of *P. flavescens* and *P. villosum* collected in late November and December exhibited, for example, what appeared to be no tetrad arrangement of microspores at all. This failure may be real, the mother-cells developing directly into the microspores, and the heterotypic division occurring at the formation of tube and generative nuclei. A homotypic division might then take place at the division of the generative nucleus. Another explanation, however, and possibly the better, is that the arrangement of microspores into tetrads is so irregular that they are not distinguishable as such; and until the development of the two-celled gametophyte one can scarcely discriminate between the diploid mother-cells and the haploid, uninucleated microspores. Instances of irregular tetrad formation in the *Spermatophytes* have been reported by Heusser (4) for *Himantoglossum*. In many anthers found in winter material there was complete disintegration of all archesporial tissue (Fig. 9). All gradations existed, however, to anthers in which there was much good pollen.

As stated above, spring and summer material exhibited variations in microspore development when compared with that gathered in winter. The principal change noted was that microspore tetrads began to appear, yet with a tendency to irregularity in arrangement which did not hinder, however, easy recognition of the group (Fig. 10). A comparison of these tetrads with those figured in Pisek's (9) article on *A. oxycedri* shows a striking resemblance. In certain instances there was an apparent attempt to form tetrads, but with only one normal-appearing nucleus in the group, the others being reduced in size, without separating walls (Fig. 11). Further development of such groups with the fate of the dwarf nuclei was not observed.

That the two *Phoradendron* species, *P. flavescens* and *P. villosum* can and do produce perfectly regular diads and tetrads was evident from an examination of later spring material (Fig. 12). In addition, meiotic divisions were largely simultaneous within the same anther. Thus it appears that failure to develop regular tetrads and simultaneous meiotic divisions was due to unfavourable temperatures, or to poor nutrition due

to the host being in dormant or winter condition, or perhaps to some other factor, or, more likely, to a combination of factors. The fact that one of the hosts was deciduous and the other evergreen did not seem to make very much difference.

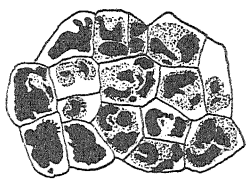


FIG. 9.

FIG. 9. *P. flavescens* var. *macrophyllum*. Portion of anther showing complete disintegration of sporogenous tissue.  $\times 200$ .

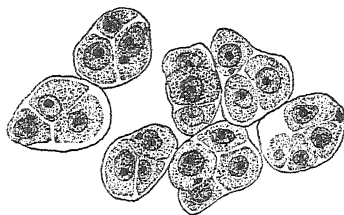


FIG. 10.

FIG. 10. *P. flavescens* var. *macrophyllum*. Microspore tetrads showing irregularities in size and arrangement of the microspores. Some of the microspores have darkly-stained granules near the nuclei.  $\times 300$ .

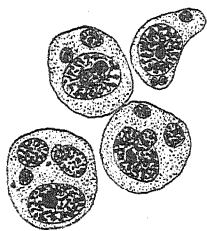


FIG. 11.

FIG. 11. *P. flavescens* var. *macrophyllum*. Tetrads with but one normal microspore nucleus and no separating walls.  $\times 450$ .

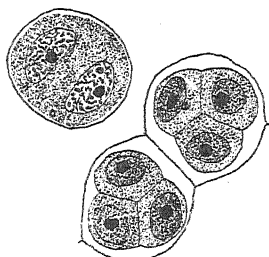


FIG. 12.

FIG. 12. *P. flavescens* var. *macrophyllum*. Diad and regularly arranged microspore tetrads. Extruded granules in the diad and one tetrad.  $\times 450$ .

In the formation of microspore tetrads there is simultaneous wall-formation following the homotypic division (Fig. 12). Schnarf (10) has published a list of species gathered from literature in which the time of wall-formation (successive or simultaneous) is given. From this list it is evident that the same type of wall-formation may not be characteristic of the different species or genera of the same family; so that the simultaneous type, as in *Phoradendron*, may not characterize necessarily other genera of the Loranthaceae. The omission of representatives of this family in Schnarf's table is doubtless due to the fact that no special mention has been made of this feature by Pisek and others.

Granules of some material staining like chromatin, and diminutive nuclei, were sometimes found in the cytoplasm during the process of microspore development. They were observed in material collected at various times and were not apparently the result of unfavourable climatic

conditions. The most constant as to size and number were two small granules that appeared in some of the heterotypic divisions (Figs. 16, 17). In other instances a larger single granule was present (Figs. 10, 12). Pisek (9), who figures a small granule or dwarf nucleus near the normal nucleus in *A. oxycedri* (his figures 3a, 3b), regards all such as partial or

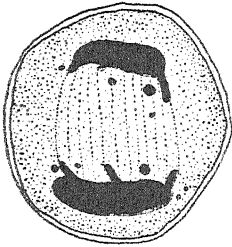


FIG. 13.

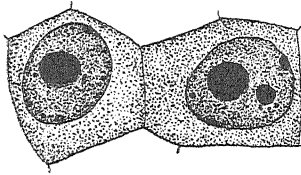


FIG. 14.

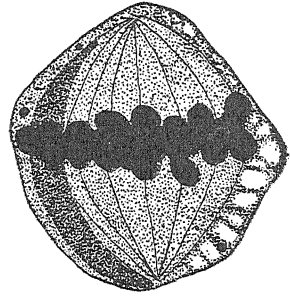


FIG. 15.

FIG. 13. *P. flavescens* var. *macrophyllum*. Late heterotypic anaphase showing granules resembling nucleoli.  $\times 675$ .

FIG. 14. *P. flavescens* var. *macrophyllum*. Microspore mother-cells showing nucleoli of various sizes.  $\times 675$ .

FIG. 15. *P. villosum*. Peculiar band of darkly-stained granules in heterotypic metaphase.  $\times 900$ .

diminutive nuclei which have taken their origin from one or more chromosomes which have remained at the equator during anaphase, or else have lost their way. In either case an independent nucleus has been organized. That the smaller granules are not from chromosomes is of course evident from their relatively very small size. Larger granules or dwarf nuclei might well have been organized from chromosomes though the transformation into such was not observed. Chromosomes which fail to reach the poles in meiosis appear in some plants as laggards on the spindle and later are seen in the cytoplasm as extruded chromatin. As no laggards during anaphase were observed in *Phoradendron*, the extranuclear chromatin, if such it be, must have been extruded at some time during prophase. One instance was observed, however, in which granules appeared both on the spindle and in the cytoplasm during a late anaphase (Fig. 13). As difficulty besets the explanation of the smaller particles as chromosomes, another conjecture is offered. It was noticed that some of the mother-cells contained within their nuclei numbers of nucleoli of considerable variation in size (Fig. 14). Should some of these be discarded in meiosis they would appear as darkly-stained granules in the cytoplasm and would be indistinguishable from those herein described as extruded. In some instances in which a single granule of larger size appears during the heterotypic division, the granule may persist till the tetrad stage and become the possession of one of the microspores (Figs. 10, 12).

A peculiar condition was found in some material of *P. villosum* gathered in June (Fig. 15). A curious spindle-shaped mass of granular matter staining black with iron-alum haematoxylin was seen to extend nearly from pole to pole in a mother-cell whose nucleus was in heterotypic metaphase. Similar cells were found in the same anther. The darkly-

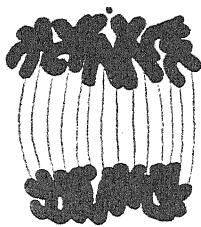


FIG. 16.

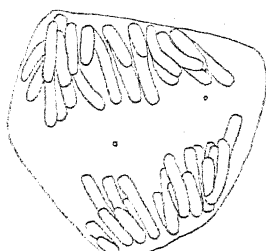


FIG. 17.

FIG. 16. *P. flavescens* var. *macrophyllum*. Heterotypic anaphase showing ten chromosomes passing to one pole and eleven to the other. Polar position of two extruded granules.  $\times 1,012$ .

FIG. 17. *P. villosum*. Heterotypic anaphase showing ten chromosomes passing to one pole and eleven to the other. Equatorial position of extruded granules.  $\times 1,012$ .

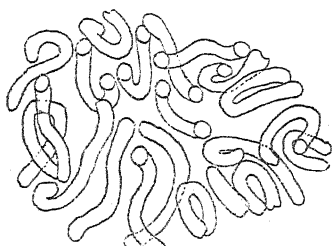


FIG. 18.

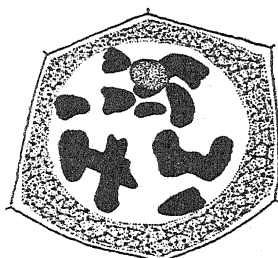


FIG. 19.

FIG. 18. *P. flavescens* var. *macrophyllum*. Somatic metaphase plate from the staminate plant. Twenty-one chromosomes.  $\times 1,350$ .

FIG. 19. *P. villosum*. Microspore mother-cell in diakinesis. Ten bivalents, and a small unpaired chromosome near the centre of the cell. The nucleolus is stippled.  $\times 1,350$ .

stained areas lay between spindle fibres and resembled what might be imagined as a coloured region between two meridians on a globe.

A count of the chromosomes during the heterotypic division in the microspore mother-cells of the staminate plant of both *P. flavescens* and *P. villosum* showed eleven chromosomes on the equatorial plate in metaphase. Upon separating, ten daughter chromosomes were seen passing to one pole and eleven chromosomes to the other (Figs. 16, 17). Evidently one chromosome did not undergo fission but passed *in toto* to the pole. The somatic count checked, yielding twenty-one chromosomes on the equatorial plate (Fig. 18). A search was then made for a nucleus in diakinesis to discover, if possible, the appearance of the odd chromosome.

Such a nucleus is seen in Fig. 19. The oval-shaped nucleolus was still present and was easily distinguishable by its form from the chromosomes. The nucleus showed ten bivalents and a narrower and shorter single

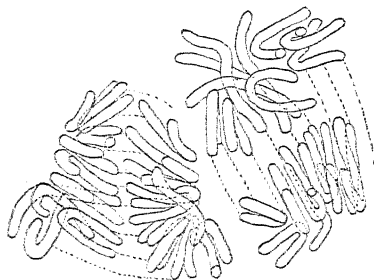


FIG. 20.

FIG. 20. *P. villosum*. Anaphases of the second division of embryo-sac development. The haploid nuclei show ten chromosomes passing to each pole.  $\times 1,012$ .

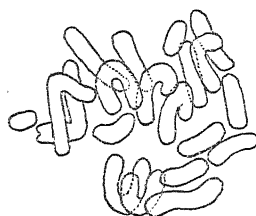


FIG. 21.

FIG. 21. *P. villosum*. Somatic metaphase plate from the carpellate plant. Twenty chromosomes.  $\times 675$ .

chromosome. In the figure this is seen near the centre of the group of bivalents. As a short element was observable in favourable views of the heterotypic anaphase in the staminate plant it was concluded that the chromosome was the same one that appeared unpaired in diakinesis.

An examination of the carpellate plant showed gametophytic anaphases in embryo-sac development with ten chromosomes passing to each pole (Fig. 20). A somatic count yielded twenty chromosomes on the metaphase plate (Fig. 21). This signifies that the small chromosome in the staminate plant is the sex determiner for that plant, and its absence is the sex-determining factor for the carpellate plant.

Our knowledge of the chromosome sex-mechanism in plants has been clearly summed up by Sharp (11), Blackburn (1), and others. These investigators divide the types of chromosome sex-mechanism according to the condition in the staminate plant into three classes as follows:—

I. The  $XY$  type in which a smaller, the  $Y$ , is associated with a larger, the  $X$ , as in *Elodea*.

II. The  $XY_n$  type in which two (or more) small  $Y$  chromosomes are associated with a larger  $X$ , as in *Rumex acetosa*.

III. The  $XO$  type in which the  $X$  is without a partner in the staminate plant, as in *Vallisneria spiralis*.

In *Vallisneria*, according to Winge (15), the staminate plant should show eight and nine chromosomes, respectively passing to the poles in the heterotypic division, making seventeen as the somatic number. It was supposed that the carpellate plant would show nine chromosomes passing to each of the poles in the heterotypic division, making eighteen as the somatic number. The  $XO$  type to be expressed fully so as to include both

kinds of plants in a dioecious species would read  $XO-XX$ . Two  $X$  chromosomes appearing in the carpellate plant give it one more than the staminate. As only two plants have been reported exhibiting the  $XO$  type, a species of *Vallisneria* (Winge (15)), and one of *Dioscorea* (Meurman (6)), evidence of the occurrence of this type among plants rests upon the findings of these investigators. But as indicated by Blackburn, there is some doubt as to this evidence for reasons outlined in her paper, so that there is a possibility that the  $XO$  type as interpreted above may have no known representatives in the plant kingdom. As the only  $XO$  type recorded thus far either among plants or animals is expressed by the formula  $XO-XX$ , it is of interest to note that in *Phoradendron* there exists a new kind of chromosome sex-mechanism, at least a new variety of the  $XO$  type which may be designated as  $XO-OO$ . In this the  $X$  chromosome does not appear at all in the carpellate plant, thus giving it one less chromosome than the staminate instead of one more in accordance with the accustomed interpretation of the  $XO$  type. Hence it appears that for the purposes of clarity the use of the formula  $XO$  alone should be discontinued and that with it should be coupled the chromosome complement of the carpellate, or female, individual as has already been the practice with Wilson (14) in describing the chromosome sex-mechanism for the animal, *Protenor*. The former  $XO$  type would then resolve itself into the *Protenor* and possibly *Vallisneria* type, expressed as  $XO-XX$ , and the *Phoradendron* type, expressed by  $XO-OO$ .

The chromosome numbers (N) for some of the species of Loranthaceae are as follows:—

Species.	N.	Authority.
<i>Viscum album</i> . . . . .	10	Pisek (8)
<i>Loranthus europaeus</i> . . . . .	9	Pisek (9)
<i>Arceuthobium oxycedri</i> . . . . .	13	Pisek (9)
<i>Dendrophthora opuntioides</i> . . . . .	9-10	York (17)
<i>Denirophthora gracile</i> . . . . .	9	York (17)
<i>Phoradendron flavescens</i> var. <i>macrophyllum</i> . . . . .	10	Billings
<i>Phoradendron villosum</i> . . . . .	10	Billings

During the maturation of the pollen-grain, the extine undergoes thickening in certain portions which gives it something of the appearance of a trefoil (Fig. 22). In winter material, thickening may begin while the pollen-grains are still in close contact with other cells; also after the germination of the microspore to form a two-celled gametophyte (Fig. 7). Between the thickenings are thin places through which the pollen tube may emerge. In a stained preparation of mature pollen there is seen a nearly spherical, more darkly-stained nucleus, the tube nucleus, and a slightly elongated, more easily decolourized, generative nucleus (Fig. 23).

In material collected even in the most favourable growing period, anthers exhibited a considerable percentage of empty, shrivelled, or

otherwise defective pollen. In *P. flavescens* and *P. villosum* it amounts to forty or forty-five per cent. In *P. californicum* it may run as low as thirty per cent. In all three species, however, some anthers show a complete abor-

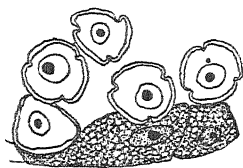


FIG. 22.

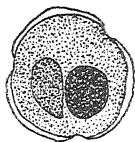


FIG. 23.

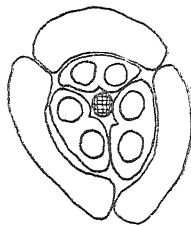


FIG. 24.

FIG. 22. *P. flavescens* var. *macrophyllum*. Microspore showing trefoil-like thickening of the walls. Stippled cells are tapetum.  $\times 300$ .

FIG. 23. *P. flavescens* var. *macrophyllum*. Mature pollen-grain.  $\times 1,125$ .

FIG. 24. *P. villosum*. Diagram of cross-section of staminate flower at microspore mother-cell stage. Two archesporia are seen in each anther.  $\times 30$ .

tion in pollen development. Pisek (9) calls attention to defective pollen in *A. oxycedri*, and thinks it is due to an unfavourable condition as to food supply which interferes with the reduction division, also produces variation in the chromosome number. While there is nothing in the external appearance of either *P. flavescens* or *P. villosum* to indicate that the parasites are poorly nourished in the spring and summer seasons, inasmuch as vigorous young shoots appear and the plants have a healthy green colour, yet there may be a sufficient degree of defective nutrition that in some way would be reflected in the more delicate reproductive processes. Bad pollen has of late been associated with irregular meiosis which is thought to indicate hybridism. In cases where there is known hybridism and the reduction division has been studied in their connexion, the irregularities as manifested by lagging or extruded chromosomes, variation in chromosome number, polyploidy, &c., are more definite and pronounced than is the case with the species of *Phoradendron* under investigation. Hence it seems, perhaps, unwise to assign to any single cause, defective and aborted pollen and other abnormalities that are associated at times with microsporogenesis, but merely to record them.

#### SUMMARY.

1. *P. flavescens*, Nutt., var. *macrophyllum*, Engelm., and *P. villosum*, Nutt., show floral development during most of the year. The desert species, *P. californicum*, Nutt., blossoms in the early spring.

2. Staminate and carpellate plants are generally in close association with consequent facility of pollination.

3. The archesporial tissue arises from the division by a periclinal wall of a single hypodermal cell in the young anther. The inner cell thus cut

off becomes the primary sporogenous cell. The tapetum originates by periclinal walls in the inner parietal layer.

4. Winter material exhibited no simultaneous heterotypic divisions within the same anther, nor regular tetrads of microspores. In this material some of the mother-cells function as tapetal cells though there is a well-developed tapetum.

5. Spring and summer material showed simultaneous heterotypic and homotypic divisions without lagging chromosomes in anaphase, but sometimes with what appeared to be extruded nucleoli or chromatin. Regular tetrad grouping of the microspores follows the homotypic division.

6. Heterotypic division in the staminate plant shows ten chromosomes passing to one pole and eleven to the other thus making the plant disporic, with a chromosome count of twenty-one in its somatic nuclei. In the heterotypic division of the carpellate plant, ten chromosomes pass to each pole, the somatic nuclei containing twenty chromosomes.

7. It is suggested that the expression  $XO$  as the designation of a type of chromosome sex-mechanism be discontinued, as it represents the chromosome complement of but one plant only in a heterophytic species. As a substitute it is suggested that the chromosome sex-mechanism formula should include the chromosome complement of both plants. Thus the *Protenor-Vallisneria* type would be expressed as  $XO-XX$ , and that of *Phoradendron* as  $XO-OO$ .

8. The cell-walls that follow homotypic divisions and cut off the individual microspores arise simultaneously.

9. Mature pollen-grains contain a two-celled male gametophyte. Defective pollen is found in all anthers and varies in amount from complete abortion of the sporogenous tissue to thirty per cent. in *P. californicum*. A common percentage of defective grains in *P. flavescens* var. *macrophyllum* and *P. villosum* is forty to forty-five.

10. The species of *Phoradendron* examined have shown considerable variation in some of the steps in microsporogenesis. Variation in chromosome number, however, does not seem to occur.

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## Contractile Roots.

### II. On the Mechanism of Root-contraction in *Oxalis incarnata*

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With Plate XL and four Figures in the Text.

SINCE the publication by one of us of an account of the contractile roots of *Oxalis incarnata*, with an explanation of the mechanism of their contraction (9), several papers dealing with contractile roots have appeared or have come for the first time to our notice.

Rohde (7) has described those of *O. esculenta* and other species, including *O. incarnata*, and has offered his own explanation of their mechanism. Berckemeyer (1) has examined the root-stocks of certain Umbelliferae and takes quite a different view of their contraction from that put forward by de Vries (13) in his classical contribution on the subject.

For Monocotyledons, Gravis (4) has given a convincing account of the contractile roots of *Crinum capense*, illustrated by Bouillienne, which obviously belongs to the characteristic type described by Rimbach (6) and Pfeiffer (5) has briefly described and figured those of *Gladiolus*, which belong to the same general type.

On the other hand, Smith's description of the contractile roots of *Brodiaea lactea* (8) makes it clear that in this Monocotyledon the mechanism of contraction is very similar to that in *O. incarnata* and quite different, in the later stages of the process, from the ordinary monocotyledonous types. Quite as remarkable in their specialization to a similar mode of contraction are the polystelic roots of certain orchids described by Fuchs and Ziegenspeck (2, 3).

The explanation offered, in the previous paper, of the process of contraction in *O. incarnata* appears to us, notwithstanding Rohde's criticisms,

to be the only one that fits the facts, and to be applicable also to certain other species of *Oxalis*, to *B. lactea*<sup>1</sup> and other species of this genus, and to Fuchs and Ziegenspeck's Dactylorchids. All of them have two features in common: (1) the absence of any longitudinally continuous contractile tissue; (2) the presence of transverse zones of cells which collapse as the root shortens (called by Fuchs and Ziegenspeck 'Puffergewebe'), alternating with turgid zones which hinder transverse contraction.

We have had under observation examples of all these types (10). In this paper, however, we propose to confine ourselves to some supplementary observations on *O. incarnata* and a consideration of Rohde's views and criticisms.

#### 1. FURTHER OBSERVATIONS ON *ONALIS INCARNATA*.

Since the first paper was published some matters on which further information was thought desirable have been made the subject of investigation. They include the question whether growth in diameter and contraction in length ever coincide.

The observations previously described showed clearly that the greater part of the process of contraction is not correlated with swelling but takes place after the root has reached its maximum diameter. They did not, however, suffice to exclude the possibility of an initial temporary phase of growth-contraction. A repetition under improved conditions was therefore undertaken.

##### *Measurements of Contraction in Length and Growth in Diameter.*

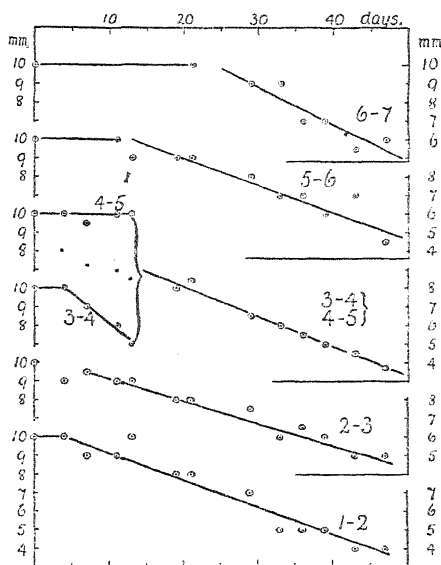
Bulbils were planted early in December, 1927, in specially constructed boxes, provided with one sloping glass side, darkened with a zinc cover. The cover and glass were readily removable for marking the roots and, if necessary, for making observations and measurements. In this way a number of healthy plants were obtained which developed strong contractile roots, and these contracted normally and extensively. A majority of these were formed close to the glass. The contraction in length and changes in diameter were followed, by measurement of marked zones, at more frequent intervals than previously.

The position of the base of each bulbil was marked on the glass on January 12, 1928. These marks served as base-lines from which the positions of bulbils and of marks on the roots could conveniently be measured. The roots were marked on February 16, by which time the upper ends had already swollen and begun to contract.

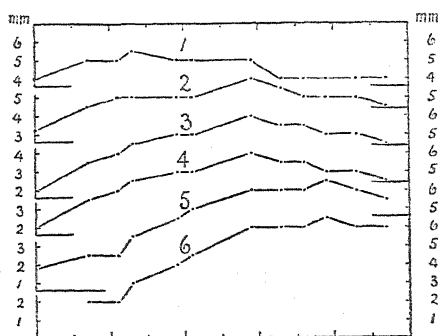
One of the plants (Plant 1 in Pl. XL, Fig. 1) produced a long straight root especially suitable for marking and measurement. The results from

<sup>1</sup> On the case of *Brodiaea lactea* see Thoday (9).

this root are represented in Text-figs. 1 and 2. Text-fig. 1 gives the changes in length of centimetre zones, between marks numbered con-



TEXT-FIG. 1. Changes in length of successive 10 mm. zones of contractile root of Plant I, between marks numbered from top of root downwards.



TEXT-FIG. 2. Changes in diameter of the same root as Text-fig. 1, at the numbered marks.

secutively from above downwards. The uppermost mark, numbered 1, was made 0.4 cm. below the base of the bulbil. In each zone, contraction appears to proceed at a fairly uniform rate once it has begun.<sup>1</sup>

As more and more of the root takes part in the process the rate of descent of the bulbil increases, as shown for this and other roots in Text-fig. 3. Ultimately, of course, the rate diminishes again. This stage was

<sup>1</sup> After the first fortnight the position of mark 4 became confused. The curve thereafter represents the average of the lengths of two adjacent zones.

only reached after the detailed measurements were stopped (April 3), but it is illustrated in Text-fig. 3 for Plant I, for which records of the position of the old bulbil are available up to May 10.

The actual rates of descent vary very much from root to root, especially with the length of root participating. The highest rate measured was that for Plant I between April 3 and 18, viz. 3.3 cm. in fifteen days or 2.2 mm. per day. The corresponding rate for the shorter root of Plant IX was 0.9 cm. in fifteen days, or 0.6 mm. per day.

For individual centimetre zones of Root I the rates of contraction vary between 0.12 and 0.2 mm. (excluding the initial period of zone 3-4), i.e. 1.2 to 2 per cent. of the initial length, per day, averaging 0.16 mm., or 1.6 per cent. per day.

The curves in Text-fig. 2 record the changes in diameter of Root I at the different marks. They illustrate how growth in thickness, like contraction, starts at the top and is initiated downwards later and later. It will be observed that no further growth in thickness was recorded after the twenty-ninth day. Contraction in length, nevertheless, continued without interruption throughout the measured region for a further eighteen days and was still proceeding then. During this period, therefore, contraction was not correlated with swelling.

On the other hand, close comparison of the two sets of curves shows that contraction may begin before growth in thickness has finished. This is clearest for zone 5-6, in which swelling was not complete till the twenty-ninth day, although it had contracted in length 2 mm. in the course of the previous fortnight.

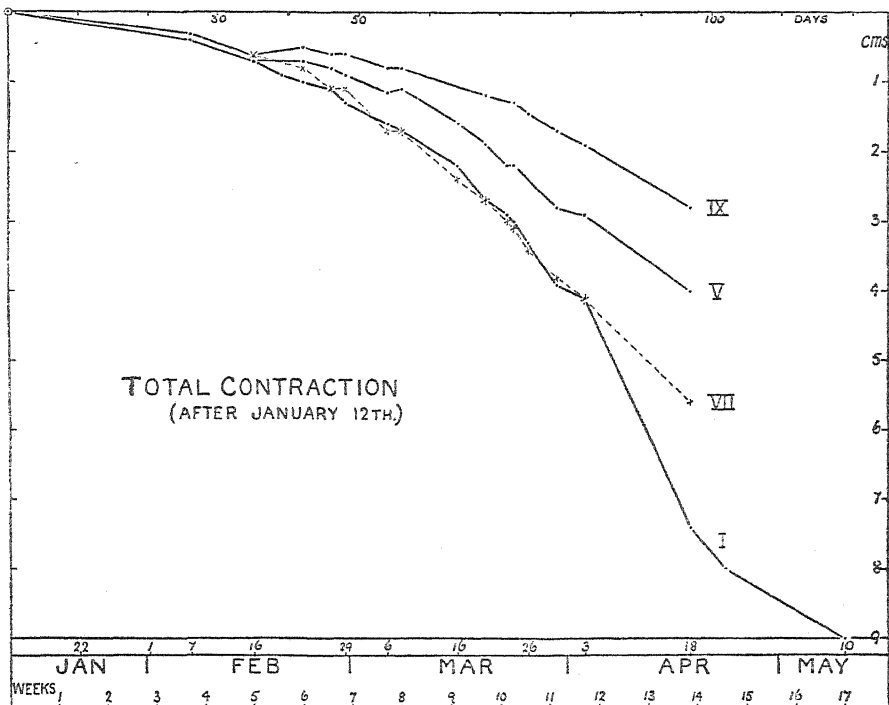
In zone 2-3 the overlapping of swelling and contraction is also clearly shown. It will be observed, however, that at mark 2 swelling ceased on the eleventh day, but that after another ten days a further expansion occurred, which is paralleled at all the lower marks, and is followed at 2, 3, and 4 by a corresponding fall. This might possibly be regarded as a reversible turgor expansion, though it is large (20 per cent.) for such an explanation to be readily acceptable. In any case, the coincidence of this renewed expansion with contraction is of doubtful significance, because in zone 2-3 the collapse of transverse zones of cells had almost certainly already begun. On the other hand, during the earlier stage of expansion from 3 mm. to 5 mm. diameter the correlation of contraction with swelling is unambiguous.

The measurements of other roots gave in various degrees the same kind of results and confirm the occurrence of contraction in these roots before they have finished their growth in thickness. Contraction in length at that time cannot be explained in the same way as the later phase of the process. This early phase must be one of true growth-contraction.

It thus appears that in *O. incarnata*, as recorded by Smith also for

*B. lactea* (8), there are two phases of contraction, each presenting distinctive features which indicate that the mechanisms of the two are quite different.

The growth-contraction process requires a contractile tissue, continuous



TEXT-FIG. 3. Curves of total contraction for four roots, of Plants I, V, VII, and IX of the culture shown in Plate XL, Fig. 1.

longitudinally, under tension, therefore straight. These conditions are fulfilled in the earlier stages while the root is growing in thickness. Our observations suggest that contraction does not invariably occur at this stage. When it does occur, longitudinal sections show the cambium straight, the older xylem elements sinuous (Pl. XL, Fig. 3). In the later phase, the cambium and the xylem core as a whole become sinuous and distorted, and the turgid parenchyma interrupted. Growing-contraction can no longer be effective. The cells of the erstwhile contractile tissue, in so far as they enlarge at all, grow vertically as well as transversely; and they no longer form a longitudinally coherent tissue. They are gradually, layer by layer, depleted in their solutes and their water. The process is one of longitudinal collapse and shrinkage.

It is not improbable, however, that growth-contraction may play a subordinate part during the latter phase. The straightening of the cambium in a fully contracted piece of root has already been commented upon (9). The capacity of the cambium of dicotyledonous contractile roots to

accommodate itself to the contraction is clearly very remarkable. It is a phenomenon which has not yet received the attention it deserves nor any satisfactory explanation.

Locally, too, under some conditions, the contraction of portions of the young parenchyma might actively assist the contraction of the root; but the distortions observed exclude the possibility of this being more than a minor contributory factor.

## 2. ROHDE'S VIEW OF THE CONTRACTILE MECHANISM.

Rohde's study of root-contraction in the genus *Oxalis* (7) was based primarily upon *O. esculenta*. The dauciform root of this species does not, it appears, lend itself so well as the longer, less tapering one of *O. incarnata* to the detailed measurement which we have been able to apply to the latter.

In a general way Rohde's anatomical observations confirm our own. He describes for *O. esculenta*, with cytological details, the development of the tissues and the disappearance of the protoplasm from transverse layers of 'buffer-tissue' in the secondary phloem, which collapse gradually between layers of cells which maintain their turgescence. He states that he was able, by the use of Flückiger's reagent, to localize reducing sugar and found it absent from those cells which could no longer be plasmolysed, while present in the cells of the turgescent layers.

His account differs from that previously given of *O. incarnata* (9) in its emphasis on three points, viz., the radial growth of the turgescent layers, their increasing obliquity, and the shearing, not crumpling, of the walls of the collapsing cells which, he maintains, are pushed over, so that their vertical walls first slant and finally become parallel to the approximating turgescent layers. On these points he bases a view of the mechanism of contraction (our later phase) as a growth phenomenon, which is ingenious but, we think, unsound.

He mentions three contributing mechanical factors:

(1) The transverse layers, alternately turgescent and flaccid, abut within on the core of cambium and xylem, outside on a zone of smaller inactive cells, bounded by the periderm. The turgescent layers by their radial expansion bulge the outer resistant sheath, which is held back between them by the passive collapsing layers and so becomes transversely wrinkled and therefore shortened longitudinally.

(2) Since the outermost tissues impede the radial growth of the turgescent layers, these tend to curve, in order to obtain room for expansion. Each layer thus bulging downwards<sup>1</sup> presses upon the layer of

<sup>1</sup> If we understand him aright, Rohde explains the *downward* curvature as being due to the fixation of the bulb in the ground, so that it is easier to compress the xylem and pull the bulb down than to push it up against the weight of soil.

'buffer-cells' below it and gradually presses the water out of it. In addition to this, the curvature of the successive active layers displaces them in relation to one another, so causing the obliquity of the tangential walls of the intervening buffer-tissue and bringing the active layers closer and closer together.

(3) Further growth of the active layers accentuates this relative displacement and the consequent flattening of the buffer-cells.

These, as we understand him, are the main points of Rohde's theory. He appears to deny that water is withdrawn from the root by the rest of the plant. The turgescient layers, during their further growth, draw on the water in the collapsing cells which are compressed between them.

We may concede at once that, if the turgescient layers grow transversely against the resistance of the periderm, a small degree of contraction might perhaps ensue according to Rohde's first mechanical factor, by wrinkling of the periderm. The following experiment may be quoted in illustration. Into a piece of rubber tubing 55 mm. long with an internal diameter of  $7\frac{1}{2}$  mm. and wall about  $1\frac{1}{2}$  mm. thick, eight discs were inserted and evenly distributed, each  $10\frac{1}{2}$  mm. in diameter and 2 mm. thick. The resulting profile was undulatory and the length was 51 mm.: thus the tube had contracted in length about 7 per cent., but there was no external resistance to overcome.

A periderm as extensible as Rohde's view assumes would yield to longitudinal as well as transverse extending forces. Moreover, our measurements on *O. incarnata* show clearly that, were any contraction brought about in this way, it could only be transitory, as the diameter tends to diminish as contraction proceeds.

Factors (2) and (3) go together, and their effectiveness would again depend in part on whether transverse growth and longitudinal contraction coincide in time and place. We cannot find in Rohde's measurements any data which demonstrate that a given *region* of the root of *O. esculenta* is ever found to be at one and the same time increasing in diameter and contracting in length.

The measurement of marked zones on roots of *O. incarnata* previously published (9) gave no evidence that longitudinal contraction and transverse swelling were correlated, but pointed rather to the opposite conclusion.<sup>1</sup> We have now established such a coincidence for *O. incarnata*, but at a time when collapse of the 'buffer-cells' has not yet begun. To render Rohde's

<sup>1</sup> Rohde's criticism that the contracting region also grows in diameter, but that the fact is obscured by the increasing obliquity of its layers is not valid. At the early stage of contraction represented by the example quoted, the obliquity is slight. The fact that at a later stage, if the oblique layers were replaced horizontally, the diameter would be 30 to 40 per cent. greater than it *is*, is not proof that it would also be 30 to 40 per cent. greater than it *was* when it began to contract. In *O. incarnata*, when obliquity becomes pronounced the diameter of the root diminishes very markedly. From this point of view, Figs. 2 and 3 in (9) Plate XVII are not comparable.



description of the process at all plausible, it would be necessary to demonstrate the coincidence not only of contraction in length and increase in diameter, but also collapse of transverse zones of cells in the same part of the root.

Factors (2) and (3) depend also on the increasing obliquity of the turgescient layers. In *O. incarnata* the obliquity is not pronounced till a late stage of contraction. Even supposing, however, for the sake of argument, that in *O. esculenta* the obliquity were early in its incidence, Rohde's theory would not work; for since the turgescient layers could curve downwards only by compressing the xylem-core (as well as the buffer-cells), *they would necessarily exert at the same time an equal and opposite extending force on the peripheral tissues* (against the action of his first factor).

In fact, Rohde's view fails entirely to account for the contraction of the root as a whole. His first mechanical factor might shorten the periderm a little, but his second factor would extend it. The more the xylem-core were compressed the more would the outer tissues be stretched.

No growth-contraction mechanism can be effective unless a shortening tissue is present capable of supporting a longitudinal tension. Instead of that, the distorted xylem,<sup>1</sup> the transverse 'buffer-zones', the wrinkled periderm, all give evidence that they are being longitudinally compressed. Rohde's own figures make it difficult to take his views very seriously. Even for *O. esculenta* his figures 5 and 7 do not show that regularity which his mechanism would seem to demand, while in *O. lasiandra* he refers to the 'weniger guten Abgrenzung der Einzelgewebe' (7, p. 516-7). In a root with the structure shown in his Fig. 9 of *O. incarnata* none of his factors would work. He recognizes (p. 519) that his second and third factors could not apply to the root of *O. cummingi*, represented in his Fig. 10, and attributes contraction in this case to his first factor; but there seems no reason why our explanation should not apply equally well to all four species.

What Rohde has apparently failed to realize is that during contraction there is a diminution of volume. It may be that this fact was obscured in *O. esculenta* by growth of the lower part of the root while the upper part contracted; but in *O. incarnata* a contracting zone may shorten, without increase in diameter, by as much as 75 per cent. In one case the whole of the swollen part of a root contracted more than 75 per cent., drawing the bulbil down more than 4 cm. Sometimes several centimetres of root reach their full diameter before contraction has proceeded far, and swelling then ceases to progress downwards, while contraction proceeds to its full extent. That is, the contracting part of the root may lose a half to three quarters

<sup>1</sup> Rohde in reference to this (7, p. 486), gives '*sehr gut mit der Anordnung eines umgebenden aktiven Verkürzungsgewebes vereinbar ist*' as a rendering of 'is hardly compatible with', &c., and so inverts the meaning of the passage quoted (9, p. 573).

of its water. Whither does it go, and how? On this question Rohde's only contribution is to ask how the solutes can be removed through living cells, the protoplasm of which is impermeable to these same substances in their own sap. Since the position of the phloem as the food-conducting tissue is now fully established<sup>1</sup> the obvious explanation is that the solutes are transferred from the collapsing cells by the slender phloem strands that traverse the parenchyma. Rohde's interpretation of these as tannin-sacs (7, p. 486) in the case of *O. incarnata* is certainly erroneous.

### 3. THE CONDITIONS FOR WITHDRAWAL OF WATER.

*Rohde's objections.* Rohde's criticisms are not all easy to follow. Some of them, if we understand them aright, imply serious misconceptions.<sup>2</sup>

His objection (p. 522) that the irreversibility of contraction is against its being a cohesion phenomenon does not hold. It has already been explained (9, p. 576) why the collapse of the dead cells must be irreversible. If the soil were saturated with water, and atmospheric conditions prevented transpiration, some slight relaxation of the collapsing cells, of periderm and of xylem, might occur; but, in general, if the collapsing cells contain no solutes, fluctuations in the water deficit would merely cause the rate of contraction to fluctuate.

A similar misconception is involved when Rohde says (p. 497), 'Es kann keine Rede davon sein, dass die Pflanze Wasser aus der fertig gebildeten Rübe sauge und also den Speicher aufzehre. Im Gegenteil, die unteren Teile füllen sich während der Kontraktion mit Wasser an.' (Similarly, p. 490). Growth in other parts, even of the root, is quite compatible with removal of water, even by cohesion forces, from the contracting part. Since the latter does diminish in volume, it must lose water. Whether it loses it to the lower part of the root, if this is still swelling, to the intercalary region of the stem (*infra*, p. 1003), or to the transpiring shoot system, is immaterial. The growth of vacuolated cells involves the development of suction pressures, whether by secretion of osmotically active solutes into the cell-sap, or by active changes relaxing the tension in the cell-wall. If a water deficit exists, however caused, and the dead cells lose their solutes, the conditions are fulfilled for the removal of water from the latter. A fuller consideration of these conditions has been published elsewhere (10).

*Air-spaces.* We have been entirely unable to confirm Rohde's statement that air-spaces are present in the parenchyma in *O. incarnata*. No trace of air can be detected in sections mounted in water and viewed by

<sup>1</sup> When the previous paper was written, this was still in doubt, after Dixon's critical consideration of the available evidence.

<sup>2</sup> E.g. (in parenthesis, p. 491) 'Wie ist es möglich, dass verwelkte Blätter transpirieren?', or (p. 448) '... ein Kohäsionsmechanismus kann nie bis zur Grösse des Turgors saugen, da der Kohäsionszug mit Auflösung der Membranspannung sein Ende erreicht.'

transmitted light. But even were they present they would not invalidate our view of the contraction. The pressure of the air in the spaces and any cohesion tension within the cells would, as Rohde says, cause the walls there to bulge inwards and they would have to bear the strain. If, in other species, air-spaces are present, the evidence shows that they are capable of bearing the strain.



TEXT-FIG. 4. Radial strip of tissue from a beet root, originally 7.05 cm. long and 6 or 7 mm square, bounded at each end by periderm, after being boiled, leached, and allowed to dry in the laboratory. The parenchymatous tissues have collapsed during drying till the volume is only a very small fraction of the original volume.

*Experiments on shrinkage.* The shrinkage of drying tissues is enough to demonstrate that air does not readily enter cells and that collapse of tissues may be very considerable.

A transverse slice of beet root, 3 cm. square and about 5 mm. thick, left to dry in the laboratory, contracted to an irregular shape, owing to the resistance of the vascular tissues, but with maximum dimensions  $23\frac{1}{2}$  mm. tangentially and 16 mm. radially. As the outside became hard and incrustated with dried solutes, a narrow prism 6 or 7 mm. square and 7.05 cm. long was cut radially, boiled in water and left to soak in water overnight to allow the bulk of the solutes to escape. It was then blotted and left in the laboratory to dry. It contracted in length to about 2 cm. (i.e. less than 30 per cent. of the original length) and at the same time the soft tissues between the zones of wood completely collapsed together. To test the pull which contraction might exert is not possible in such a case, but it has been measured for the annulus of a fern sporangium by Ursprung in hundreds of atmospheres (12).

*Differentiation.* We are also unable to confirm Rohde's statement that in the swollen root there is already, before contraction begins, a pre-existing differentiation of smaller buffer-cells from rather larger 'active' cells. The obliquity of the radial walls gives sometimes an appearance which might be so misinterpreted (cf., e.g., (9) Pl. XVII, Fig. 3), but tangential sections show no such alternation until contraction is beginning.<sup>1</sup> Nor can we find any structural or cytological differences when the parenchyma is first formed. The differentiation of collapsing cells is, in our view, not structurally predetermined but is physiological. The fact that 'active zones' later become additional 'buffer-zones' confirms us in this view.

<sup>1</sup> Rohde exaggerates the regularity of the alternation in the contracting region when he says (l.c., p. 518) 'Es folgt bei dieser Art exakt eine aktive Zone aus einer Zelllage auf eine Pufferzell-schicht', and (p. 527) 'in einschichtige Zelllagen aus aktivem Gewebe und ebensolchen aus Puffer-gewebe sorgfältig differenziert ist'.

# FURTHER PROBLEMS.

It is scarcely necessary to emphasize that we do not regard the process of contraction as automatic. Everything points to the production of contractile roots, the direction of their growth, and their subsequent contraction, as each being initiated or determined in response to stimuli, though the nature of these stimuli has still to be demonstrated. Even in the later phase of contraction, it is the active changes in the metabolism of the 'buffer-cells' which constitute the immediate response, leading to depletion of solutes and disappearance of protoplasm, which in turn prepare the way for those forces which actually bring about the contraction. Correlated with contraction is the intercalary elongation of the stem immediately above it. A whole complex of phenomena is thus presented, towards the causal analysis of which we have but made a contribution.

As illustrating some of the problems involved, the photographs in Pl. XL are of interest. Fig. 1 shows the experimental box of plants already referred to, as it appeared when opened up on April 24, after four months' growth, and three weeks after the detailed measurements were stopped.

It illustrates very well the intercalary elongation of the stem which allows the root to contract without disturbing the aerial shoot-system. The correlation between the two phenomena, elongation of the stem and contraction of root, is remarkable; but it is not perfect. In several of the plants shown, intercalary growth of the stem has exceeded contraction and the stem has become sinuous. In Plant VIII the reverse has happened: the stem has grown too little and the aerial shoot has been pulled down a couple of centimetres.

The extent of contraction of the roots of these plants is extraordinary. The original position of the old bulbils is easily recognizable by the thicker bases of the aerial stems, about on a level with the top of the swollen root of Plant III. Most of the roots in the photograph have already withdrawn far towards the bottom of the box. The part hidden by the zinc base (3 cm. deep) was largely occupied by potsherds.

The swollen part of Root IX, which included all the marks used in measurement and was therefore more than 4 cm. long (probably about 5 cm.) was at the time of the photograph 2.1 cm., and three weeks later (May 10) only 0.9 cm. long. The top of the root of Plant VIII (hidden in the photograph) is within a centimetre of the top edge of the zinc. Plant I is that for which measurements have been given in Text-figs. 1 and 2. The top of the root had descended 7.4 cm. on April 18, a week before the photograph was taken. By May 10 it was 9 cm. down. The total distance from the original position of the bulbil to the very bottom of the box was only about 12 cm.

On April 24 the plants were in full flower and only beginning to form

bulbils, but the roots were already well down. Later examination showed that their elongation had been limited by the bottom of the box. A deeper box of the same pattern was therefore constructed and bulbils planted in it to see what the limit might be to the depth of descent and how it would be established. The culture was less successful, as the bulbils did not all grow and the roots formed away from the glass; but two plants grew to a flourishing maturity. Fig. 2 shows one of them uncovered when the flowering period was well advanced. The top of the root was about 13 cm. below the soil-level, and only 3 or 4 cm. remained unswollen. Near the still undamaged tip lateral roots had formed. The maximum diameter was at least 7 mm., greater therefore than any in the previous experiment. Contraction, however, seems not to have been anywhere as energetic a process as in the other experiment, and may already have come to a standstill; for the upper 6 cm. had shrunk in diameter by nearly a half, and nowhere is the wrinkling, indicative of longitudinal contraction, so prominent and vigorous as it is in the roots shown in Fig. 1. The comparison suggests that the longer the root the less vigorous the contraction of any part of it. Further experiments must show whether this is so, as interpretation is complicated by the fact that the first lot were grown during the early part of the year and the second in the summer months, so that other factors may have influenced the results.

The plant in Fig. 2 had already produced a fine crop of bulbils, a few scattered along the intercalary stem, but most of them developed from the subterranean part of the upward-growing stem. The latter has apparently been pulled down a couple of centimetres, and from near its base an oblique, branched swollen root has grown, similar to a contractile root in appearance and diameter and doubtless capable of contracting. The formation of a second contractile root is still better shown by Plant III in Fig. 1, in which the uncontracted root had grown from the base of the thicker upward part of the stem, while the principal root, seen a little to the right, had already contracted strongly.

Besides the problems presented by these correlations, their mechanisms and determining factors, there are anatomical problems. The changes in form of the cells of the contracting parenchyma, in the early phase of contraction, are difficult to envisage adequately. When first formed they have a characteristic lozenge shape in tangential section which changes to a more or less isodiametric oblong. The cambium seems to show little change in its average dimensions, notwithstanding the contraction of the root. How it adapts itself is a problem that has not, so far as we are aware, been faced. For its solution a larger and more uniform supply of roots in different stages of development will be required than we have yet succeeded in growing.

# SUMMARY.

1. New measurements of the changes in length and thickness of marked zones of contractile roots of *O. incarnata* have revealed a temporary phase of growth-contraction preceding the more extensive contraction, by collapse of transverse layers, previously described.

2. Rohde's explanation of contraction in *Oxalis* and his objections to the explanation previously put forward are critically considered, with special reference to the conditions governing shrinkage by withdrawal of water.

3. Observations are described illustrating the correlations which await elucidation, and attention is drawn to some other outstanding problems.

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## EXPLANATION OF PLATE XL.

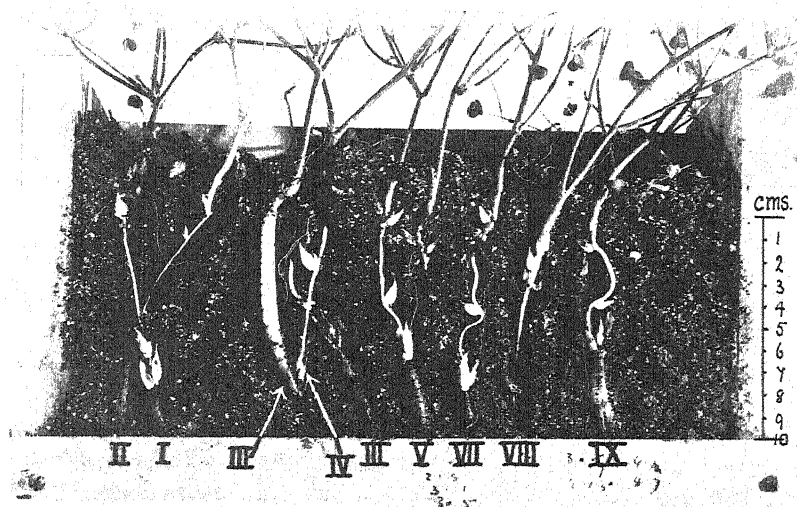
Illustrating Professor D. Thoday and Miss A. J. Davey's paper on the Mechanism of Root-contraction in *Oxalis incarnata*.

Fig. 1. Box of plants, with sloping glass side and zinc cover both removed, to show roots in various degrees of contraction, slender intercalary stem, new bulbils, &c. Plant III has formed a second contractile root.  $\times$  about  $1/2$ -8.

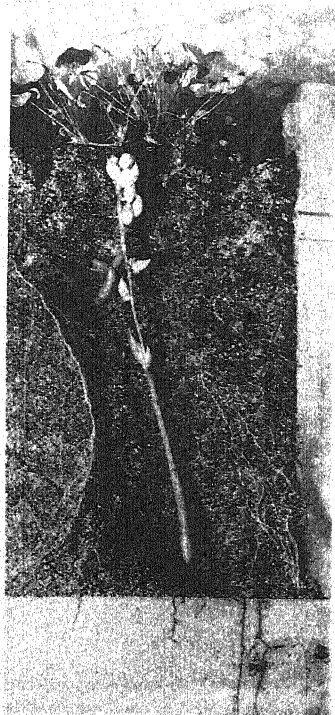
Fig. 2. Plant in deeper box, with greater length of root less vigorously contracted, a branched lateral swollen root, and numerous bulbils.  $\times$  about  $1/4$ .

Fig. 3. Longitudinal section of root in early phase of contraction. The phloem parenchyma shows no collapsing layers, the cambium and young vessels are straight, but the old xylem strand is sinuous.  $\times$  108.





1



2



3





# Studies in the Inheritance of Physiological Characters.

## II. Further Experiments upon the Basis of Hybrid Vigour and upon the Inheritance of Efficiency Index and Respiration Rate in Maize.

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With five Figures in the Text.

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### I. INTRODUCTION.

IN discussing the significance of the efficiency index in plant growth, V. H. Blackman wrote in 1919 (3, p. 358): 'For the highest production of vegetable matter by the single plant, two factors are necessary—large seeds, and a high economy in working represented by a large efficiency index. The importance of these two factors in breeding cereal crop-plants should be borne in mind; it may be possible to breed for a high efficiency index.'

Blackman conceived of the growing plant as accumulating dry weight at 'compound interest' for the duration of the grand period of growth.

The efficiency index, or relative growth rate, is a measure of the rate of interest, and is the value of  $r$  in the equation:

$$W_t = W_0 e^{rt}$$

where  $t$  = time,  $W_0$  = initial weight,  $W_t$  = weight of the plant after time  $t$ , and  $e$  is the base of Napierian logarithms.

In the first paper (I) of this series an example was given of the importance of embryo size in determining the production of vegetable material and in the manifestation of hybrid vigour, and the data collected gave some hint of a regular mode of inheritance of the efficiency index. The growth rates of two strains of maize and their  $F_1$  hybrid were studied over a period of seventy days. The hybrid exhibited remarkable vigour in this instance. It was shown that this vigour was not to be explained by the presence of a greater efficiency index in the hybrid; in fact, the efficiency index of the hybrid was exactly the same as that of one of the parents. The results indicated that the greater size of the hybrid was the result of the possession of a larger embryo than either of its parents, so that vigour in this hybrid was nothing more than the maintenance of an initial advantage in embryo size. The work suggested a number of problems as to the nature of hybrid vigour and the inheritance of the efficiency index. It is the purpose of this second paper to present data for the solution of some of these problems. The data, which relate to maize, are presented in three groups: (1) data on the segregation of the efficiency index in the  $F_2$  population; (2) data on the manifestation of hybrid vigour in reciprocal crosses; (3) data on the respiration rates of parents and of a hybrid strain.

## II. THE INHERITANCE OF EFFICIENCY INDEX.

It was established in the first paper (I) of this series that the efficiency index of the hybrid population of maize examined was the same as that of one of its inbred parents, while it differed considerably from that of the other. It was pointed out that this might be an instance of complete dominance in the  $F_1$  population of a factor or complex of factors determining the efficiency index.

The results might have been peculiar to the particular strains examined; moreover, they were obtained in England, where the climate is unsuitable for the growth of maize. For these reasons the experiments were repeated in Chicago, using inbred lines of maize totally different from those used in the previous work. In addition, the analyses were carried to the  $F_2$  generation, to discover whether there was any segregation of the efficiency index.

The material was provided by Mr. F. D. Richey, of the United States Department of Agriculture, from his breeding stocks. It comprised two lines which had been self-fertilized for nine generations, one  $F_1$  cross between

these lines, and the  $F_2$  of the cross, obtained by sib-pollinating the  $F_1$  plants. Mr. Richey's pedigree for the parent lines were 228-6-5-Sq and 228-4-8-Sq, designated in this paper as  $P_1$  and  $P_2$  respectively. The  $F_1$  grain had been produced on plants of  $P_2$  as the pistillate parent.

The grain was sown on 23 June, 1930, in a plot of uniform ground in Chicago. The four different types ( $P_1$ ,  $P_2$ ,  $F_1$ , and  $F_2$ ) were sown in rows 2 ft. apart, the grains being at intervals of 18 in. Each row contained only one type. The order of types was determined by shuffling and dealing four cards at random.

The summer was very hot and dry, and the plants were repeatedly watered as uniformly as possible. Along one edge of the field irregular growth took place. It was possible that the soil had a higher moisture equivalent along this edge so all plants in this region were rejected. The four types of grain germinated successfully and a uniform stand was obtained, though the viability of one parent was much less than that of the other.<sup>1</sup>

### *Sampling.*

For the two inbred parents and the  $F_1$  population the same method of sampling was followed as in the previous experiment. At regular intervals, from seven to ten plants were dug up at random and taken to the laboratory. Leaf areas were not determined; they give no indication whatever of the effective assimilating surface, since every square centimetre of the leaf is at a different angle to the incident light, and it is improbable that the assimilation of leaves of different ages is the same. The samples were dried at 110° C. for twenty-four hours. The stems of older plants were sliced into strips before drying. This method of drying the material gave figures which were satisfactory for comparative purposes. The dried plants were weighed separately, the dry weights were averaged, and the standard error of the weights calculated. The growth was studied only for the period during which the efficiency index was constant.

If there is any truth in the suggestion in the first paper that the efficiency index is inherited according to mendelian principles then a segregation should occur in the  $F_2$  population. Hence it would be useless to sample the  $F_2$  plants throughout the growth season, for one could not distinguish in a weekly sample one efficiency index from the other. The only satisfactory method was to sample the whole of the  $F_2$  population at the end of the grand period of growth. It is clear that this method of analysis would be vitiated if the  $F_2$  population were heterogeneous as to embryo size. Reference to Table II below will show, however, that this is not the case. Since, therefore, the  $F_2$  population is homogeneous as to

<sup>1</sup> Percentage germination:  $P_1$  48 per cent.  $P_2$  78 per cent.  $F_1$  82 per cent.  $F_2$  82 per cent.

embryo weight when planted, a departure from normality of the dry weight distribution on harvesting will be evidence either of heterogeneity of the soil, or of a segregation in that population of the efficiency index.<sup>1</sup>

*Data of the experiment.*

In Tables I and III the data gathered in the experiment are collected. In Table II is the result of a statistical analysis of the frequency distribution of embryo weights in the  $F_2$  population.

TABLE I.

*Grain Weight and Embryo Weight.*

Strain.	Grain.	Weights (mg.)		Scutellum.	Total.	% S.D.
		% S.D.	Embryo.			
$P_1$	192	9.38	2.23	17.5	19.73	11.1
$P_2$	248	8.88	3.57	24.41	27.98	10.1
$F_1$	251	6.38	4.47	34.85	39.32	9.22
$F_2$	343	7.28	3.65	25.44	29.10	12.1

TABLE II.

*Test of Agreement Between Frequency Distribution of Embryo Weights in  $F_2$  and Normal Distribution.*

Mean.	No. of individuals.	$\sigma$ .	$\chi^2$ .	n.	P.	Departure from normality.
3.654	49	0.381	2.728	4	0.60	not significant.

TABLE III.

*Values of Dry Weights of Samples Throughout the Experiment.*

Day.	$P_1$ .	% S.D.	Number in sample.	$P_2$ .	% S.D.	Number in sample.	$F_1$ .	% S.D.	Number in sample.
7	0.074	11.1	6	0.177	10.1	6	0.262	9.2	7
14	0.166	10.3	9	0.305	11.5	10	0.417	9.8	12
27	0.830	—	7	1.21	—	10	2.82	—	8
37	4.57	10.1	10	5.03	9.3	10	14.5	10.0	10
43	7.97	11.0	10	9.33	11.1	10	22.4	12.1	10
50	25.2	12.3	10	18.6	10.7	10	78.6	13.0	10
56	43.7	13.2	12	33.0	11.2	13	105.3	13.8	11

It will be seen from Fig. 1 that the logarithms of dry weight, for the first fifty days at any rate, fall along a straight line when plotted against time. By the method of least squares, lines of closest fit have been applied to these values, and from the equations to these lines, values for the efficiency indices of the three populations have been derived. These

<sup>1</sup> A segregation of time of germination would also produce a departure from normality of the dry weight distribution, but there is no evidence of such a segregation.

formulae are given in Table IV, and in Table V are compared the observed and calculated values of dry weight.

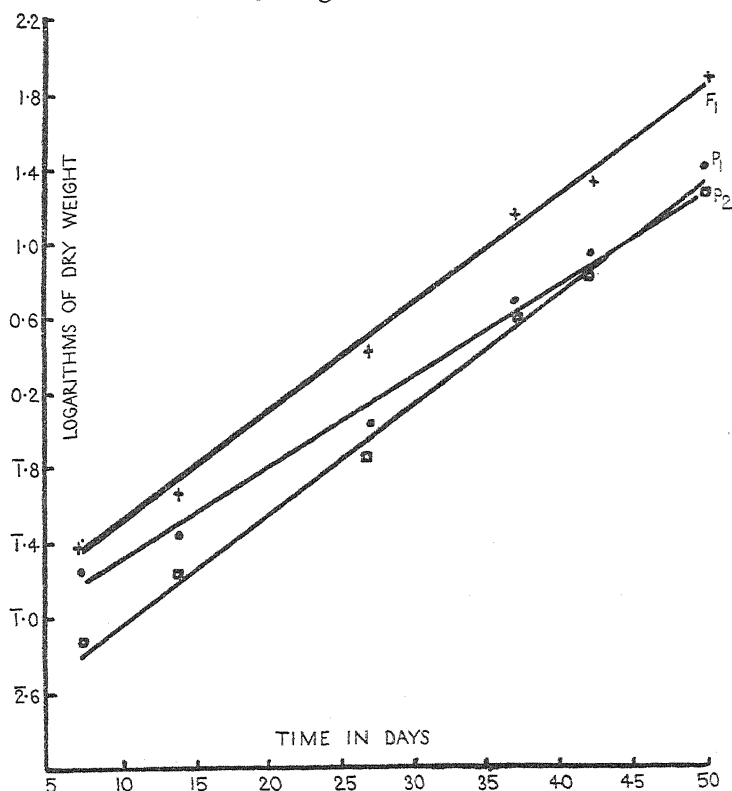


FIG. 1. Logarithmic growth curves of two parent strains of maize, and their  $F_1$  hybrid. The points represent the means of successive dry weight samples. The lines are the lines of closest fit, calculated by the method of least squares.

TABLE IV.

Strain.	Logarithmic equation.	Derived exponential equation.
$P_1$	$y = 0.0593x + 2.3895$	$W_t = W_0 e^{0.1065 t}$
$P_2$	$y = 0.0493x + 2.8380$	$W_t = W_0 e^{0.1135 t}$
$F_1$	$y = 0.0585x + 2.9360$	$W_t = W_0 e^{0.1348 t}$

TABLE V.

*Comparison of Observed and Calculated Values of Dry Weight.*

Days.	Obs.	$P_1$ Cal.	Obs.	$P_2$ Cal.	Obs.	$F_1$ Cal.
7	0.074	0.064	0.177	0.154	0.262	0.229
14	0.166	0.164	0.305	0.343	0.417	0.563
27	0.830	0.981	1.21	1.49	2.82	3.16
37	4.75	3.79	5.03	4.62	14.5	12.58
43	7.97	8.66	9.33	9.40	22.4	23.97
50	25.2	22.8	18.6	20.6	78.6	74.10
56	43.7	51.2	33.0	40.2	105.3	164.2

The agreement is sufficiently close to warrant the use of an exponential equation as a convenient summary of growth for the first fifty days. Since the curve constructed from the observed values fluctuates on either side of the theoretical curve the agreement is, in fact, closer than is indicated from the table.

The significance of the differences in slope between the three curves (i.e. the differences in efficiency indices between the three populations) can be tested, after adjustment for irregular intervals of sampling, by a method described by Fisher (5) for the comparison of regression lines. The results of these tests are summarized in Table VI.

TABLE VI.

*Comparison of Slopes of Curves for  $F_1$  and  $P_1$ , and for  $F_1$  and  $P_2$ .*

	Diff.	n.	t.	P.
$F_1$ and $P_1$	0.008	6	0.60	0.5 - 0.6 not significant
$F_1$ and $P_2$	0.099	6	12.70	< 0.01 significant

The striking phenomenon reported in a previous paper is again encountered. The efficiency indices of the two parents differ significantly. The greater of these two efficiency indices reappears in the hybrid as a completely dominant character. Although the hybrid has exactly the same efficiency index as one parent, yet on the fiftieth day of the experiment the average weight of the hybrid plants is between two and three times that of the parent. This apparent paradox is explained by the fact that the hybrids had embryos about twice as large as those of the parent, the initial advantage being maintained throughout the period of most active growth.

The apparently complete dominance of a character in the  $F_1$  generation gives little information as to the mode of inheritance of that character. Since the embryo weights of the  $F_2$  population were normally distributed (see Table II) any departure from normality in the dry weight distribution of the harvested plants must be attributed to differences in behaviour after germination; that is to say, either to soil heterogeneity, or to segregation of time of germination, or to segregation of efficiency index. It should be added that a *normal* distribution of weights in the  $F_2$  population would not necessarily indicate absence of segregation of the efficiency index; it might indicate that the index was governed by many segregating factors rather than by a few.

On account of the limited space available for drying the plants, the whole  $F_2$  population could not be harvested on the same day. The plants were collected and dried in two batches, the first on the fifty-sixth day, and the second on the sixtieth day after planting. The distribution of the weights in frequency classes of 10 grm. is given in Table VII. The distributions of the residual populations of the parents and the  $F_1$  generation

are also given. Since the populations were harvested on different days, their average weights are not comparable, for they represent different periods of growth.

TABLE VII.

*Frequency Distribution of Dry Weights of Maize Populations.*

	$P_1$ .	$P_2$ .	$F_1$ .	$F_{2a}$ .	$F_{2b}$ .
Day harvested :	61	62	65	56	60
Class means.					
gram.					
24.95	—	1	—	—	—
34.95	—	1	—	1	—
44.95	2	3	—	1	1
54.95	5	13✓	—	9	1
64.95	14✓	6	—	3	9
74.95	6	4	—	4	4
84.95	4	1	—	10✓	2
94.95	4	2	2	1	11✓
104.95	—	—	4	2	4
114.95	1	—	3	1	2
124.95	—	—	7	2	2
134.95	—	—	17✓	—	—
144.95	—	—	7	1	1
154.95	—	—	—	—	2
164.95	—	—	3	—	—
Totals	36	31	43	35	39

Attempts were made to fit probability curves to these frequency polygons, in order to find whether the distributions differed significantly from the normal. The results of these tests are tabulated in Table VIII.

TABLE VIII.

*Degree of Conformity of Population Weights with the Probability Curve.*

Population.	Mean weight.	$\sigma$ .	$\chi^2$ .	n.	P.	Departure from normality.
$P_1$	71.1	16.31	3.95	2	0.20	not significant
$P_2$	60.5	15.82	4.50	2	0.10	not significant
$F_1$	130.0	16.93	3.30	2	0.20	not significant
$F_{2a}$	78.15	25.28	12.83	3	0.01	significant ✓
$F_{2b}$	91.63	26.63	9.17	3	0.028	significant ✓

The distributions of the dry weights of the parents and the  $F_1$  population do not differ significantly from normal frequency curves. The distribution of the  $F_2$  population, however, is significantly different. Since the  $F_2$  population was interplanted with the other populations an abnormal distribution due to soil differences should have been common to all the populations. This is not the case. There was no measurable difference in the rate of germination of the two parents, so that one would not expect such a factor to segregate in the  $F_2$  generation. One is led to the



conclusion that a segregation of the efficiency index has undoubtedly occurred, though the data are too meagre to indicate in what ratios the factors have segregated. It may be tentatively concluded that the efficiency index is governed by relatively few factors, which segregate out to give an abnormal distribution in the  $F_2$  generation.

### III. HYBRID VIGOUR IN RECIPROCAL CROSSES.

It is commonly recognized that the grain weights of reciprocal crosses of maize differ considerably on account of the influence of the two maternal nuclei in the formation of the triploid endosperm. The embryo, however, receives but one set of chromosomes from both father and mother, and reciprocal crosses therefore give embryos of the same genotypic composition. Moreover, according to the current mendelian hypothesis of hybrid vigour, whereby vigour is attributed to the assembling of complementary dominant factors in the hybrid, the vigour of reciprocal crosses should be the same. Material to test this hypothesis was not available from the maize sent to the writer in 1929. Accordingly, in 1931, material of two inbred parents and their reciprocal crosses was obtained from Mr. F. D. Richey, of the United States Department of Agriculture. For convenience, the parents will be referred to as  $P_3$  and  $P_4$ , and the hybrids as  $F_1(P_3\varphi)$  and  $F_1(P_4\varphi)$ .

The first experiment to be performed was extremely simple. Grain of the parents and the reciprocal crosses was soaked at room temperature for twelve hours. After this period the embryos were dissected out from every grain. The scutellum was separated from every embryo, and the remaining part of the embryo dried for an hour at  $100^\circ\text{C}$ . and weighed. The object of this experiment was to obtain a measure of the capital of meristematic cells with which the embryo begins its life as an autotrophic plant. The scutellum does not undergo cell division, nor does it become part of the adult plant; it was therefore dissected away before the embryo was weighed. It is considered that the weight of the remaining part of the embryo is the best measure which can be obtained of the original capital which subsequently accumulates at compound interest. The results of the experiment are given in Table IX.

TABLE IX.

*Dry Weights (mg.) of Embryos and Grain of Maize Populations.*

Population.	Grain.	Embryo.	S.E.
$P_3$	250	2.66	0.314
$P_4$	221	3.57	0.318
$F_1(P_3\varphi)$	321	3.78	0.339
$F_1(P_4\varphi)$	329	4.29	0.379

In Table X are given the results of a statistical analysis of the differences in embryo weights.

TABLE X.

Populations.	Diff.	t.	n.	P.	
$P_3$ and $P_4$	0.91	6.0	16	< 0.01	significant difference
$F_1(P_3 \varphi)$ and $F_1(P_4 \varphi)$	0.41	3.1	25	< 0.01	significant difference

The embryo weights of the two parents differ significantly, as do also the embryo weights of the reciprocal crosses.

The first fact to be deduced from these figures is that there is no necessary correlation between embryo weight and grain weight. In fact, in the table above, the heavier parent grain possesses the lighter embryo. This would be sufficient to explain the contradictory nature of data on the influence of seed weight on yield, quoted by Kidd and West (9). As an estimate of the meristematic capital of the embryo, grain weight is worthless.

The second fact is that there is a significant difference between the embryo weights of the reciprocal crosses although the reciprocal crosses have the same genetic constitution. This is clear evidence that the difference is due to a maternal effect during the development of the embryo and before the seed enters upon its resting period. On this hypothesis the parent  $P_4$  provides better conditions for embryo development than the parent  $P_3$ .

Since hybrid vigour in these strains signifies nothing more than the maintenance of an initial advantage in embryo size, these reciprocal crosses should exhibit different degrees of hybrid vigour. The following experiment was performed to test this point.

Populations of the two parents and the reciprocal crosses were germinated between sheets of blotting paper at 25° C. When the embryos were of sufficient size they were transferred to quart milk bottles containing culture solution.<sup>1</sup> About seventy seedlings in all were grown. The experiment was conducted in a greenhouse, and temperature records were kept by means of a thermograph. Every five days every plant was removed, dried by pressing lightly between wads of blotting paper, weighed, and put back into fresh culture solution. The operation upon one plant occupied about three minutes.

In this way curves of increase in wet weight for every individual plant were obtained. The parent  $P_3$  germinated very irregularly and many of the leaves withered. The data from this population were therefore discarded. Grain of the other parent and the reciprocal crosses germinated well and produced healthy plants. The experiment was continued for twenty-five days.

<sup>1</sup> 1 grm.  $\text{KNO}_3$ ; 0.5 grm.  $\text{CaHPO}_4$ ; 0.25 grm.  $\text{MgSO}_4$ ;  $\text{FeCl}_3$  trace;  $\text{H}_2\text{O}$  1 litre.

The data were treated in the following way. The logarithms of wet weight were plotted against time for each individual plant. In nearly

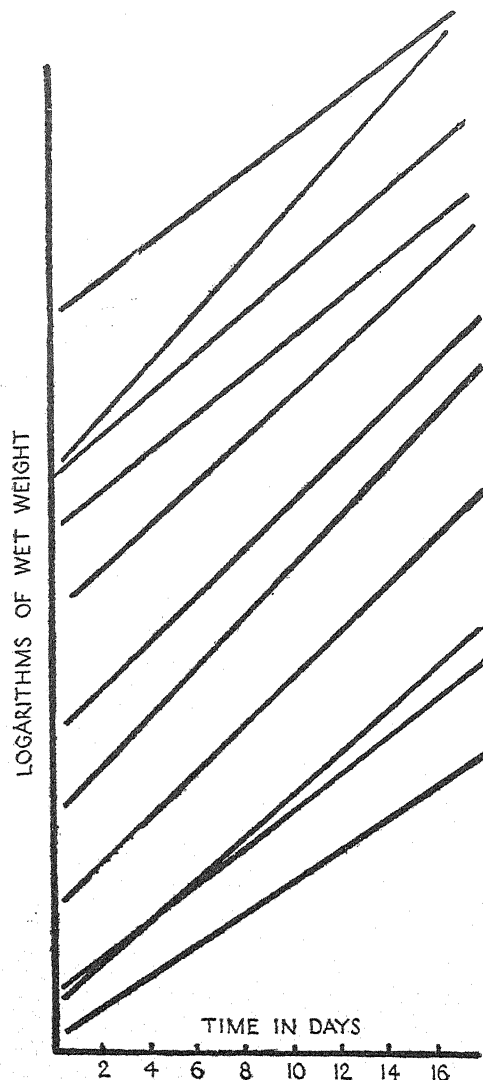


FIG. 2. Logarithmic growth curves of wet weight for every individual plant in the population  $F_1$  ( $P_1 \times P_2$ ). Each curve has its own zero on the ordinate, in order to avoid confusion on the graph; and for the same reason the points through which the curves were drawn are omitted. From the slopes of these curves the mean efficiency index of the population was calculated (see Table XI).

every instance the values fell along straight lines, indicating that the efficiency indices had remained constant. The data from those plants whose efficiency indices had not remained constant were discarded, data from about five plants being rejected on this ground. Data for forty-nine

plants remained, and through the plotted logarithms of wet weight for each plant there was drawn by eye the straight line of closest fit. It was not necessary to employ the method of least squares since the values deviated very little from straight lines. In this way forty-nine logarithmic growth curves were obtained (Fig. 2); the tangents of their angles of slope ( $\tan A$  in the Tables XI and XII) were taken as a measure of the efficiency indices of the plants. These tangents were measured graphically. A statistical treatment of the results will therefore cover errors due to variation in the efficiency indices themselves, and also errors involved in the graphical estimation of the efficiency indices.

The data from the experiment are too extensive to be recorded in detail, but the results are summarized in Tables XI and XII, and in Figs. 2 and 3.

TABLE XI.

*Data from Water Culture Experiment on Maize. The Populations were Germinated on 2nd May and Harvested on 27th May, 1932.*

Strain.	Plant number.	Mean wet weight. gram.	% S.E.	Mean $\tan A$ .	% S.E.
$P_4$	21	3.16	11.60	0.8421	7.49
$F_1(P_3 \times P_4)$	11	4.56	17.40	0.8606	8.95
$F_1(P_4 \times P_3)$	17	5.55	13.03	0.8456	6.52

( $\tan A$  is the tangent of the angle of slope of the logarithmic curve.)

TABLE XII.

*Test of Significance of Differences between Reciprocal Crosses, &c.*

	n.	t.	P.	
Difference in wet weight of reciprocal crosses after 25 days . . . . .	0.99	26	4.56	< 0.01 significant
Difference in efficiency indices ( $\tan A$ ) of reciprocal crosses . . . . .	0.150	26	0.347	0.70 not significant
Difference in efficiency indices of $P_4$ and $F_1(P_3 \times P_4)$ . . . . .	0.185	30	0.422	0.60 not significant

It is clear that the reciprocal crosses show different degrees of hybrid vigour in that their wet weights after twenty-five days' growth differ significantly, but their efficiency indices show no such difference. It is to be noticed that the efficiency indices of the hybrids are the same as that of the parent  $P_4$ , a corroboration of the results obtained earlier in this paper. Unfortunately no reliable data are to hand as to the efficiency index of the other parent  $P_3$ .

Fig. 3 illustrates these results graphically. The three logarithmic curves are parallel to one another within the limits of accuracy of the experiment, i.e. the efficiency indices of  $P_4$  and of the two reciprocal crosses

are the same. The relative differences in weight at the end of the experiment are the same as the relative differences in weight at the first sampling; in other words, the greater vigour of one hybrid over the other is nothing

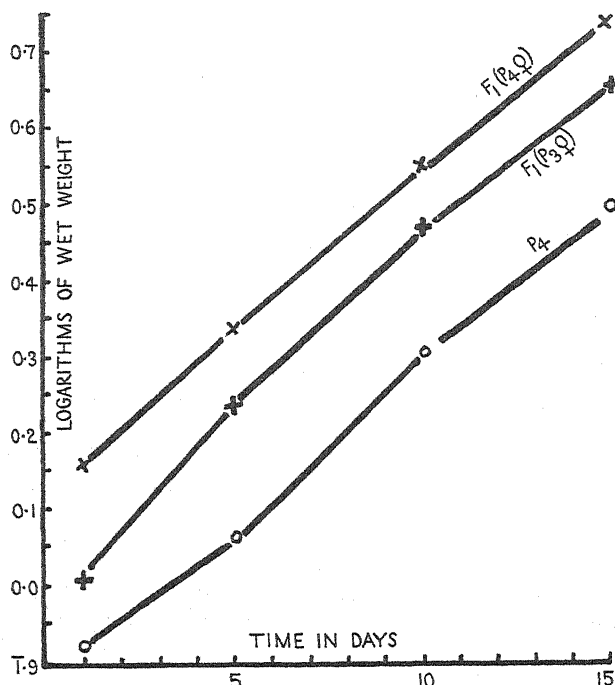


FIG. 3. Logarithmic growth curves of a parent and two reciprocal crosses. The points represent the means of the wet weights of the total populations.

more than the maintenance of an initial advantage in embryo weight. Reference to Table IX shows that the hybrid with the greater vigour had the heavier embryo. It is reasonable to conclude that the difference in hybrid vigour between the reciprocal crosses is due to a difference in the embryo weights of their grains. It should be added that the correspondence is not absolutely exact: the embryo weights were in the ratio 1/1.13, and the final wet weights in the ratio 1/1.22.

Since the reciprocal crosses have the same genetic constitution, one would expect them to have the same efficiency index, and this in fact is the case. Further discussion of these results will be found on p. 1027.

That such minute differences in embryo size should be responsible for relatively big differences in final weight is a striking example of the compound interest law in biology. Increase in final yield of dry matter has often been accomplished by increasing the efficiency index experimentally, but the writer can trace no experiments designed to increase the 'capital' of dividing cells in the embryo by influencing during maturation the final embryo size in the resting seed.

## IV. HYBRID VIGOUR IN VERY YOUNG SEEDLINGS.

The experimental results indicate that hybrid vigour in the strains of maize studied is the outcome of a larger embryo in the hybrid grain. Although the larger store of endosperm associated with the hybrid grain

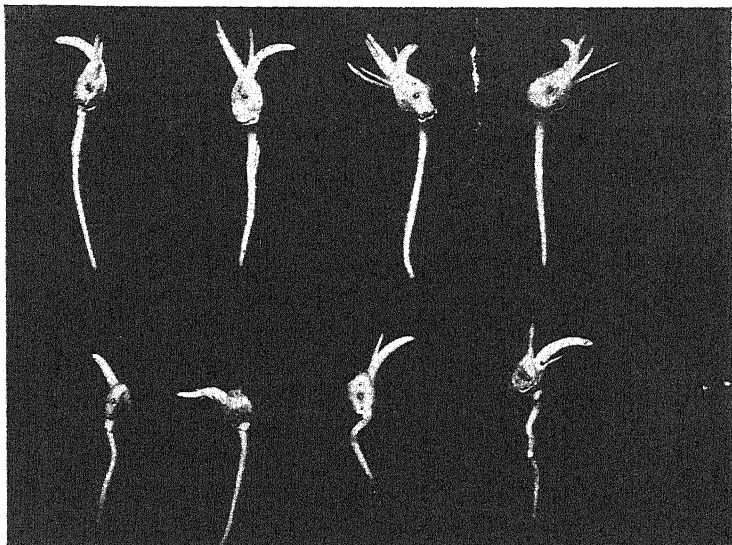


FIG. 4. Samples of seedlings of maize from which the endosperms have been dissected, grown in distilled water and in darkness. The seedlings in the upper row are  $F_1$  hybrids. Those in the lower row are from the  $P_4$  parent population with the same efficiency index as the  $F_1$  hybrid. Hybrid vigour is evident in the  $F_1$  seedlings.

may be contributory to the subsequent vigour, a very simple test demonstrated that hybrid vigour occurs independently of the influence of the endosperm.<sup>1</sup>

Grain of the parent  $P_4$  and the hybrid  $F_1(P_4\phi)$  was soaked in water for ten hours at room temperature. At the end of this period the endosperms were removed and the seedlings germinated in darkness at  $25^\circ\text{C}$ . between sheets of blotting paper soaked with distilled water. There was, therefore, no external nutrient supply to the seedlings. In the next forty-eight hours they lost in weight but increased considerably in volume. In Fig. 4 are shown examples of seedlings from the two strains. The upper row is from hybrid grain, and the lower row from parent grain. There is even at this early stage very evident hybrid vigour. The plumules of the

<sup>1</sup> A comparison of the ratio of embryo weights of parent and  $F_1$  populations with the ratio of the dry weights of those populations on harvesting (p. 1018) would indicate that differences in amount of stored foodstuffs play very little part in determining differences in yield of vegetable material. The indications are that the capital of meristematic cells rather than the capital of stored foods is the determining factor in yield of vegetable material.

hybrid were no longer, though their mean diameter was greater. The most striking manifestation of hybrid vigour is in the number of lateral roots which have appeared. The greater number of meristems in the roots of hybrids of maize as compared with their parents has often been noticed by the writer in the course of his experiments.

#### V. RESPIRATION RATES OF PARENTS AND OF HYBRIDS OF MAIZE.

Before summing up the results of these experiments on hybrid vigour and efficiency index in maize some experiments on respiration rates in maize which have a bearing upon the question of hybrid vigour will be described.

One of the possible hypotheses to account for the greater weight of the hybrid plants in these strains of maize involves the respiration rates of the hybrids. For if the relative respiration rate of the hybrid were less than those of the parents, and the relative rate of photosynthesis the same, the hybrid would accumulate dry matter more rapidly than its parents. While this, if it were true, would be at variance with the hypothesis of hybrid vigour put forward above, it seemed profitable to test it by direct experiment.

In the first paper of this series (I) some evidence was advanced in support of the view that the respiration rate did not differ significantly from that of either parent. There was not, however, sufficient material to test the matter critically. Unfortunately, the strains of maize used in the first experiments (1) could not be obtained; but by courtesy of the United States Department of Agriculture, the writer was able to obtain two inbred parents which were sibs of the strains used before, together with one of the hybrids between them. Grains of the one parent ( $P_w$ ) were white and large, and of the other parent ( $P_b$ ) were blue and crinkled. Grains of the  $F_1$  were pale blue and larger than those of either parent. All three populations had been harvested in 1928 at the same time and under the same conditions from the farm of the Department of Agriculture.

The mode of experimentation was very simple. The respiration rates of embryos of the two parents and their hybrid were measured, to discover whether there was any systematic difference between them. Measurements were made in a respirometer of the Warburg-Barcroft type, by determining the pressure change in manometers as the carbon dioxide of respiration was absorbed.

##### (a) *Apparatus.*

A standard Warburg-Barcroft respiration apparatus, with six respiration vessels and manometers, was found to be most convenient for the work. The vessels were of a suitable size and shape to hold maize seed-

lings about forty-eight hours old, and the carbon dioxide evolved in respiration was absorbed by potash solution in a side vessel. It was possible to measure a change in volume of the system of 0.001 c.c.<sup>1</sup>

The manometers and vessels were calibrated by filling with mercury and weighing. For every vessel a 'factor' had to be calculated, whereby pressure changes could be translated into volume changes. In calculating the factor allowance was made for 0.5 c.c. potash, 4.0 c.c. water, 0.2 c.c. cotton wool (on which the seedling rested), and as a first approximation, 0.3 c.c. for the seedling itself. The liquid in the manometers was Brodie's fluid,<sup>2</sup> of which 10,000 mm. are equivalent to a pressure of one atmosphere. The water bath could be kept constant to within 0.04° C. Since there was in every experiment a blank manometer small changes in temperature were immaterial. For further details as to the manipulation of the apparatus the reader is referred to Warburg's paper (14).

(b) *Preliminary experiments.*

Before the data required could be collected, a number of preliminary experiments had to be carried out.

1. Since the respiration is measured by the absorption of carbon dioxide in potash, it was necessary to determine the respiratory ratio of maize seedlings. To ascertain this, germinating seedlings were left in the manometer vessels, without potash, for fifty hours. In no case was a significant change in the pressure to be observed. It was concluded that over this part of the life cycle the ratio of carbon dioxide evolved to oxygen absorbed was unity.

2. Before an arbitrary measure of respiration for comparison was chosen the course of respiration through the first fifty hours after soaking was followed. It was thought that the curves in time of the respiration of the two parents might differ. In Fig. 5 are given curves of respiration, in c.c. oxygen per hour, of single  $P_b$  and  $P_w$  seedlings, from the time of soaking in water. All the curves obtained were of this general shape. It will be noticed that readings can be taken three hours after the dry seeds are soaked. Although the absolute respiration rates of the two seedlings were very much the same in the instance cited, the weights of the embryos differed considerably, so that the relative respiration rates differed. It was concluded from this test that the general course of development of the three types of seedlings was the same for the first fifty hours.

It was the aim of the investigation to discover whether there is any difference in intensity of respiration between the three populations. To accomplish this a large number of determinations had to be made, and it

<sup>1</sup> The apparatus can be obtained complete from J. Emerson, Brattle Street, Cambridge, Mass., U.S.A.

<sup>2</sup> 23 grm. NaCl; 5 grm. sodium cholate; 1 drop alcoholic thymol; 500 c.c. water.



was impracticable to measure the respiration in every instance over a period of fifty hours. Consequently the amount of respiration at some arbitrary time after germination was chosen as the criterion. It was decided to

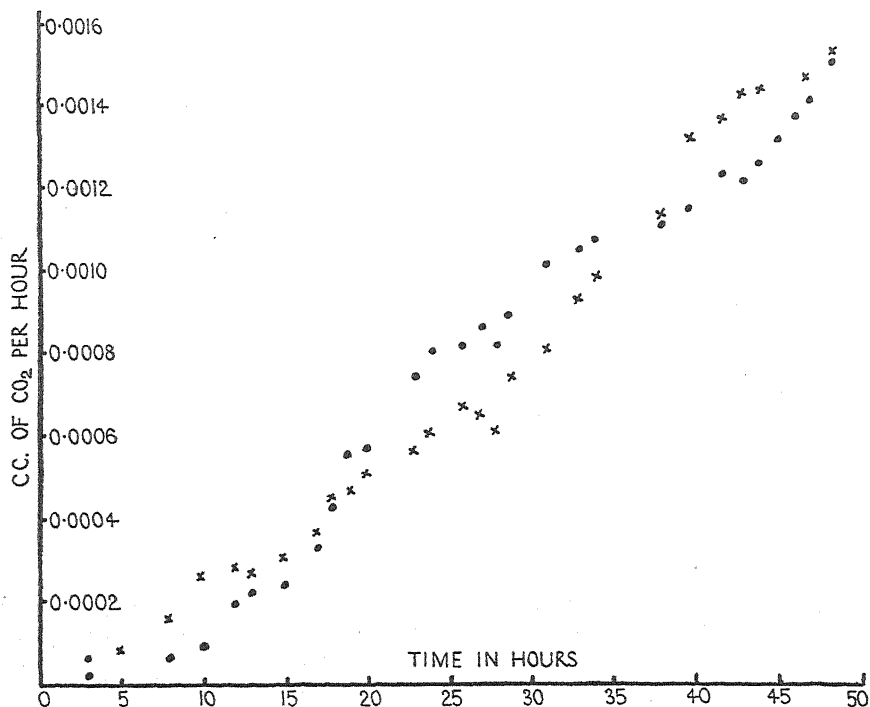


FIG. 5. The respiration rate, in c.c. carbon dioxide per hour, of one seedling of each of two inbred stocks of maize. It will be observed that readings were taken three hours after the grain was soaked.

measure the respiration from the forty-eighth to the fiftieth hour after soaking.

3. The values obtained for respiration are meaningless unless referred to some standard. The best standard is the amount of protoplasm in the embryo, but the method of measuring protoplasmic nitrogen is not above criticism and would have absorbed too much time. As an arbitrary standard to which respiration could be referred there was adopted the dry weight of the embryo and scutellum, dissected out from the grain at the end of the experiment fifty hours after germination.

4. Early experiments were unsatisfactory on account of occasional contamination by fungi, detected by a rapid increase of respiration in the chambers. This was avoided by sterilizing the seeds in uspulun. Preliminary tests showed that previous treatment with uspulun did not alter either the respiration rate or the rate of growth of the seedlings.

(c) *Method of experimentation.*

The method of conducting an experiment was as follows: At 9.30 a.m. about twenty maize grains were soaked in uspulun for twenty minutes, washed in sterile water, and put between damp filter papers in a sterile Petri dish. The dish was then put into an incubator at 25° C. After forty-eight hours, i.e. at 9.30 a.m. two days later, the dish was removed from the incubator and five seedlings, as uniformly developed as possible, selected by eye. The stage of growth chosen was that after the appearance of the main root, and before the appearance of lateral roots. The five selected seedlings were put one into each of the five manometer vessels. The sixth vessel contained as a control a seedling killed by immersion in boiling water.

The manometers were left open to the air for thirty minutes, by which time the seedlings had reached the temperature of the water bath and manometers. The manometers were then set, closed to the atmosphere, and the absorption of carbon dioxide measured over the period of one hour by the change in volume every fifteen minutes. At the end of this period the seedlings were removed from the vessels. The embryos and scutella of the seedlings were dissected out, put on watch glasses, dried in vacuo at 70° C. for an hour, and finally weighed.<sup>1</sup> In this way when germination had been satisfactory, and enough embryos were available, five determinations could be carried out at once, and the respiration values in every instance were the means of four or five readings.

In Table XIII is given an example of a morning's readings, and in Table XIV all the results are summarized. Table XV embodies a statistical analysis of these results.

It will be observed that there is a significant difference between the respiration rates of the two parents, on a basis of their dry weights, and that the respiration rate of the hybrid population, on the same basis, follows that of one of the parents ( $P_b$ ) and differs significantly from that of the other parent ( $P_w$ ). The difference between the respiration rates of the two parents is, however, small.

Although the respiration on a basis of dry weight is an arbitrary measure and its genetical significance somewhat obscure, one can conclude from the experiments that the respiration rate differs in the two parents, and that the respiration of the hybrid follows that of one of the parents. Hybrid vigour in this hybrid, therefore, is not the result of any decrease in the relative respiration rate of the hybrid.

<sup>1</sup> Such a method of drying applied to delicate material has already been found satisfactory (13).

TABLE XIII.

Grain (*F*<sub>1</sub>) soaked 9.30 a.m. 25.2.30.

Respirometer 10.00 a.m. 27.2.30.

The factors for manometers 2-6 are 0.00915, 0.00976, 0.00900, 0.00823, and 0.00815 respectively.

Manometers:— Time.	Control.		2.		3.		4.		5.		6.	
	h.	h.	h.	d.	h.	d.	h.	d.	h.	d.	h.	d.
10.30-10.45	0.27	3.67	3.67	13.6	3.04	11.1	3.90	14.5	5.26	20.0	4.27	16.0
10.46-11.01	0.22	3.67	3.67	13.8	2.98	11.1	4.02	15.3	5.26	20.5	4.14	15.7
11.02-11.17	0.02	3.43	3.43	13.7	2.70	10.8	3.74	14.9	5.07	20.5	3.87	15.5
11.18-11.33	0.12	3.53	3.53	13.7	2.87	10.9	3.80	14.7	5.26	20.5	4.07	15.8
11.35-11.50	0.07	3.50	3.50	13.7	2.87	11.2	3.80	14.9	5.07	20.0	4.07	16.0
Means.				13.7		11.1		14.9		20.2		15.8
Embryo dry weight (mg.)				0.0268		0.0241		0.0253		0.0301		0.0267
Respiration, c.c.-hour				0.126		0.108		0.134		0.166		0.129
Respiration per dry weight c.c./mg.				4.7		4.5		5.3		5.5		4.8

h. = difference in height of manometer columns, uncorrected.

d. = corrected difference, expressed in mm. per hour.

TABLE XIV.

No.	$P_b$			$P_w$			$F_i$		
	Respiration.	Dry weight.	Ratio.	Respiration.	Dry weight.	Ratio.	Respiration.	Dry weight.	Ratio.
		gram.			gram.			gram.	
1.	0.047	0.0101	4.7	0.127	0.0231	5.5	0.126	0.0269	4.7
2.	0.054	0.0121	4.5	0.117	0.0225	5.2	0.108	0.0241	4.5
3.	0.034	0.0096	3.9	0.116	0.0221	5.3	0.134	0.0253	5.3
4.	0.035	0.0105	3.7	0.148	0.0238	6.2	0.166	0.0301	5.5
5.	0.064	0.0125	5.1	0.093	0.0196	4.7	0.128	0.0267	4.8
6.	0.052	0.0116	4.5	0.118	0.0205	5.8	0.115	0.0274	4.2
7.	0.055	0.0113	4.8	0.095	0.0211	4.5	0.114	0.0253	4.5
8.	0.056	0.0121	4.6	0.101	0.0184	5.5	0.152	0.0303	5.0
9.	0.037	0.0089	4.1	0.133	0.0237	5.6	0.153	0.0373	4.1
10.	0.050	0.0097	5.2	0.141	0.0267	5.3	0.104	0.0247	4.2
11.	0.034	0.0093	4.0	0.119	0.0222	5.4	0.145	0.0301	4.8
12.	0.035	0.0092	3.8	0.120	0.0239	5.0	0.117	0.0255	4.6
13.	0.050	0.0111	4.5	0.119	0.0205	5.8	0.120	0.0268	4.5
14.	0.051	0.0104	4.9	0.105	0.0199	5.3	0.111	0.0348	3.2
15.	0.038	0.0113	3.4	0.110	0.0224	4.9	0.117	0.0259	4.5
16.	0.045	0.0087	5.2	0.129	0.0234	5.5	0.148	0.0314	4.7
17.	0.046	0.0092	5.0	0.138	0.0265	5.2	0.153	0.0295	5.2
18.	0.035	0.0086	4.0	0.109	0.0217	5.0	0.130	0.0325	4.0
19.	0.037	0.0098	3.8	0.098	0.0200	4.9	0.137	0.0333	4.1
20.	0.045	0.0101	4.5	0.111	0.0218	5.1	0.151	0.0301	5.0
21.	0.047	0.0113	4.2	0.127	0.0259	4.9	0.129	0.0335	3.8
22.	0.051	0.0109	4.7	0.150	0.0283	5.3	0.119	0.0284	4.2
23.	0.041	0.0103	4.0	0.111	0.0201	5.5	0.151	0.0321	4.7
24.	0.044	0.0092	4.8	0.103	0.0213	4.8	0.140	0.0298	4.7
25.	0.045	0.0118	3.8	0.137	0.0249	5.5	0.135	0.0306	4.4
26.	0.043	0.0111	3.9	0.146	0.0247	5.9	0.161	0.0335	4.8
27.	—	—	—	0.108	0.0215	5.0	0.109	0.0274	4.0
28.	—	—	—	0.111	0.0235	4.7	0.141	0.0320	4.4
29.	—	—	—	0.107	0.0215	5.0	0.173	0.0331	5.2
30.	—	—	—	0.120	0.0266	4.5	0.126	0.0300	4.2
Mean			4.4 ± 0.0904			5.2 ± 0.0712			4.5 ± 0.0878

TABLE XV.

	Difference in respiration rate.	$n_1 + n_2$ .	t.	P.	
Between $P_b$ and $P_w$	0.80	54	5.50	< 0.01	significant
„ $P_b$ and $F_1$	0.10	54	0.75	0.55	not significant
„ $P_w$ and $F_1$	0.70	58	6.21	< 0.01	significant

## VI. DISCUSSION.

Although the interpretation of hybrid vigour and the inheritance of the efficiency index are two aspects of the same problem, it will be convenient to discuss them separately.

(a) *Hybrid vigour.*

Three hypotheses have been put forward to account for hybrid vigour. The first claims that hybrid vigour is due to some 'stimulus' at fertilization. This view is found in many text-books on plant physiology (7), but it may be dismissed, since it merely restates the problem of hybrid vigour. The second hypothesis, originally advanced by Keeble and Pellew (8), attributed hybrid vigour to the union in the hybrid of complementary dominant characters. The third hypothesis, expounded by East and Hayes (4) is merely an elaboration of the second, taking into account the phenomenon of linkage. For a critical discussion of these hypotheses the reader is referred to East and Hayes's monograph.

The data assembled in this paper are not at variance with the mendelian hypothesis of hybrid vigour, though they require certain qualifications of that hypothesis. According to the mendelian hypothesis one would expect the efficiency index of the hybrid exhibiting heterosis to be greater than that of either parent. In the hybrids examined this has not been the case. On the contrary, the hybrids have inherited the higher of the two parental efficiency indices, and the results have shown that vigour has been nothing more than the maintenance through the grand period of growth of an initial advantage in the amount of meristematic capital, the capital being measured by the weight of the actual embryo apart from the scutellum.

The phenomenon of hybrid vigour in the instances examined is thus to be referred back to some event between fertilization and the setting of seed. The larger embryo of the hybrid may be due to an earlier division of the zygote, to a difference in the growth rate of the embryo, or to a longer period of growth of the embryo before the 'resting' of the seed. As to which of these causes operates there are no data whatever. Each of them might be attributed to a combination of favourable characters from both parents; but it is clear that an increase in embryo size, which is the basis of hybrid vigour, is determined by other influences beside particular genetic factors, for reciprocal crosses which are identical genetically possess different embryo weights. As suggested above, the other influence

in question is that of the mother plant upon the seed during maturation. The difference in vigour between the reciprocal crosses grown in the experiments described on p. 1014 is thus easy to interpret. Although the plants grew *after* germination under the same environmental conditions, the environments during their development on the mother plants, had been different. The expression of their similar genotypic constitution was therefore masked by the dissimilar parental environments in which they had lived before germination.

The same consideration would also offer an explanation for the capriciousness of the appearance of hybrid vigour. Crosses of some varieties show surprising vigour; crosses of other varieties show none at all. This capriciousness is difficult to explain from the mendelian hypothesis. But since hybrid vigour is dependent on embryo size, and embryo size is determined partly by the environment within the developing fruit—an environment which is itself very variable—capriciousness in the occurrence of hybrid vigour is scarcely surprising.

A final demonstration that the embryo size is the determining factor in heterosis in these strains of maize was provided by the exhibition of hybrid vigour in seedlings from which the endosperm had been removed, and which were grown in distilled water and in darkness.

The experiments reported in this paper have been confined to a few strains of maize, and one is not justified in extending these conclusions further, even to other varieties of maize. For, as will be pointed out below, the mechanism of hybrid vigour may differ for different plants, or for different varieties of the same plant. The writer considers these experiments to demonstrate that an initially larger embryo is *sufficient* to account for the phenomenon of hybrid vigour in maize, and further, that embryo size depends upon factors other than the genetical constitution of the ovum.

(b) *The inheritance of efficiency index.*

Attempts at a genetic analysis of yield have revealed the fact that a great many different factors contribute to it, and that yield cannot be related to any one genetic unit or group of units. It seems, therefore, that an analysis of such a complex factor is best begun by determining the mode of inheritance of the various physiological processes contributing to yield.

V. H. Blackman has pointed out that two major factors determine the yield of vegetable matter in a plant: (1) the size of the seed (or embryo) from which the plant germinates, and (2) the efficiency index. To these two factors might be added two others: the length of time for which the efficiency index remains constant, and the form of the growth curve after that time.

The next step in the analysis is to discover the manner in which all

these factors are inherited. The efficiency index depends upon the balance of anabolic and catabolic processes within the plant, upon the rate of cell division, and other processes. Of these processes nothing is known as to the inheritance of assimilation; though Beljakoff has shown (Lundegårdh 10) that the photosynthesis curves of two races of barley differ widely, and Gregory and Crowther have shown (6) that different varieties of barley differ in their manurial requirements. The respiration per unit dry weight, as has already been shown, seems to be specific for different strains, and the specific rate seems to have been inherited in one instance as a dominant in the  $F_1$  generation. Of the inheritance of rate of cell division little is known, though it has been shown that different strains of *Paramecium* have their specific rates of cell division. That meristem formation is inherited is plainly indicated by the many experiments on the inheritance of what is vaguely termed 'habit' in plants, 'habit' being a concept embracing frequency of branching, i.e. frequency in initiation of meristems.

There is some evidence, beside that adduced here, of a regular mode of inheritance of the 'constants' of growth curves. Robb, working with rabbits, found a close agreement between the  $F_1$  and one parent, as regards a constant ' $K$ ' in his equation for growth (11). Sirks (12) working on the growth rates of leaves in *Vicia faba*, concludes:

'However these strains show remarkable differences between them when compared for the steepness of the growth curve and this gives the impression of quantitative differences in the factors, which are the cause of the growth rate in the three dimensions. This impression is affirmed by the results of the crosses. In the  $F_1$  generations the growth factor of greatest intensity seems to dominate rather completely; in the  $F_2$  generations the dimensions . . . in the same individual are always the same, and the segregations suit entirely to the monohybrid 3:1 scheme.'

As a further example of the inheritance of quantitative characters reference may be made to the excellent analysis of Balls (2) for the cotton plant. Balls analysed the height of cotton plants into three dimensions, internode length (which he shows is inherited in a regular manner), growth rate, and the falling off in growth (described by Balls as 'Thermotoxy'). The  $F_1$  of a cross between an Egyptian and an Upland cotton is larger than either parent, though the internodes are no longer. No data are published on the growth in the early part of the life cycle, but in Balls' monograph, Fig. 56, is given a diagram of the curves after they have become concave to the time axis. From approximate calculations the relative growth rates of 'Sultani' and 'King' differ considerably, while the relative growth rate of the  $F_1$  closely resembles that of the parent 'King'. In his  $F_2$  populations Balls obtained frequency distributions in the form of bimodal curves, with a greatly increased variability indicative of segregation.

In the maize grain the weight of the embryo is only one-tenth the weight of the grain; but in the cotton seed the greater part of the weight is the embryo. Balls' observations on seed weight of cotton are confirmed by the writer's observations on embryo weight in maize. Balls found that the seed weight of the  $F_1$  population was greater than that of either of the parents, and in the  $F_2$  the range of weights was greater than in the  $F_1$  but the mean was lower. From the data he accumulated Balls concluded that seed size segregates in a regular manner, and is governed by a single allelomorphic pair of factors, the segregation being obscured by autogenous fluctuation. The evidence from reciprocal crosses of maize conflicts somewhat with this, but the conflict is apparent rather than real, for the maternal influence upon embryo weight, which masks the genetic influence, depends upon the parent strains selected for experiment.

Further examples of the same nature might be quoted, but these are sufficient to demonstrate that there is some body of evidence to indicate that the growth rate can be inherited in the  $F_1$  generation as a simple dominant. In all the instances studied by the writer, and in those reported by Sirks, the *higher* relative growth rate has been dominant over the lower. In the  $F_2$  generation an undoubted segregation of efficiency index occurred in the writer's population of maize, and similar segregations have been reported by Sirks and by Balls.

That the efficiency index should be inherited like many morphological characters is not surprising. Since, however, it is commonly assumed that the efficiency index is affected by many genetic factors one would not expect it to appear as a complete dominant in the  $F_1$  generation. Indeed, according to the current theory of hybrid vigour favourable factors combined in the hybrid should produce an efficiency index greater than that of either parent. This is clearly not the case with certain strains of maize.

The fact that the current theory fails to account for the behaviour of these strains of maize may arise from one of two causes: the efficiency index of one parent may be epistatic over the other, or the magnitude of the efficiency index may be controlled by a single genetic factor inherited as a dominant from either parent.

The studies of growth in this laboratory make it difficult to assume that one factor alone can control growth during the period of constant efficiency index. It would appear rather that the efficiency index under a given external environment, other than the optimum, is controlled by all the factors in the environment, and the fact that two plants may display different efficiency indices under the same set of environmental conditions indicates that their *organizations* differ. In the hybrid from these two parents both the parental organizations are present, and since the efficiency index is merely a magnitude, the highest value it can attain will naturally be that of the parent with the more efficient organization. In other words,



the efficiency index will appear *as if* determined by a simple mendelian factor, the higher efficiency index always being dominant in the  $F_1$  generation.

It must not be implied that this hypothesis is of general application to the inheritance of growth characters; it merely offers an explanation for the data offered in this paper, where a value known to be conditioned by many processes in the plant is inherited as if controlled by one or two mendelian factors only. In certain circumstances the efficiency index of the hybrid may not be identical with that of either parent. A combination of supplementary characters from both parents may result in a hybrid with an efficiency index intermediate between or greater than that of either parent; such a combination may in fact be the cause of the increased embryo weights in the hybrids of maize. Similarly a combination of parental characters which eliminates in the hybrid a deficiency factor may result in an increased efficiency index. But such data as there are support the view that no such combination takes place in the inheritance of efficiency index, but that the higher efficiency is physiologically dominant in the  $F_1$  generation, and that in the  $F_2$  generation the higher and lower efficiency indices segregate out.

*Note.*

A recent paper by M. Sirks (Beiträge zu einer genotypischen Analyse der Ackerbohne. 'Genetica', xiii. 209-631, 1932) contains interesting data relevant to the above discussion. Sirks adduces evidence in *Vicia faba* of a plasmatic influence, modifying the expression of certain growth factors. A difference in size of seeds from reciprocal crosses he attributes to an inhibitory effect on the developing embryo of the small testa from one of the maternal parents.

## VII. SUMMARY.

In the course of various experiments two inbred lines of maize were grown, together with their reciprocal  $F_1$  crosses, and in one instance the  $F_2$  generation. The plants were sampled for dry or wet weights at regular intervals. In this way estimates were obtained of the degree of hybrid vigour and of the efficiency indices of the various populations. The following conclusions were reached from the data gathered:

The  $F_1$  hybrids in all cases exhibited considerable hybrid vigour.

In every instance studied the *higher* of the two parental efficiency indices appeared in the hybrid as a complete dominant; therefore hybrid vigour in these strains could not be attributed to the possession of a greater efficiency index.

Reciprocal crosses had the same efficiency index but exhibited different degrees of hybrid vigour.

The presence of hybrid vigour without any increase in the efficiency

index of the hybrid is accounted for by the greater embryo weight in the hybrid grain. *Hybrid vigour in these strains is nothing more than the maintenance of an initial advantage in embryo size.* In other words, the 'capital' of dividing cells is greater in the hybrid, but the 'rate of interest' remains the same as that of one parent.

Although reciprocal crosses have the same genetic constitution they have different embryo weights, and this accounts for the different degrees of hybrid vigour which they exhibit. The hypothesis is put forward that embryo weight is largely determined by the influence of the mother plant during maturation of the seed.

In the  $F_2$  generation a segregation occurred of the higher and lower efficiency indices inherited from the grandparents.

Respiration rate, calculated on a basis of dry weight, appeared to be inherited in the  $F_1$  generation as a simple dominant.

The foregoing results are at variance with the current theory of hybrid vigour, and a modification of that theory is suggested to include the behaviour of the hybrids examined.

It is with pleasure that I record my thanks to the members of the Botanical Department of the University of Chicago for the facilities given me in their laboratories, and to Mr. F. D. Richey, of the United States Department of Agriculture, for his interest and advice. I am grateful also to Professor V. H. Blackman, Professor F. M. Engledow and Dr. F. G. Gregory for their guidance and criticism; and to Mr. W. Hales, Curator of the Chelsea Physic Garden, where some of the experiments were conducted.

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## Studies in the Physiology of Parasitism.

### XIII. An Analysis of the Factors Underlying Specialization of Parasitism, with Special Reference to Certain Fungi Parasitic on Apple and Potato.

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With two Figures in the Text.

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#### I. INTRODUCTORY.

IN an earlier number of this series (Vasudeva, 5) an analysis was made of the factors which restrict the parasitic range of certain fungi. The two organisms examined were *Monilia fructigena* and *Botrytis allii*, parasitic

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respectively on apple and onion tissue. The inability of *M. fructigena* to attack onion was found to be associated with the inhibitive effect of certain volatile principles of onion juice on the growth of this fungus. *B. allii* was similarly found to be non-parasitic to apple tissue, except to a slight degree in very ripe fruit, but in this case no anti-action of the host juice on the germination or growth of the fungus was demonstrable. Evidence was given that the resistance of apple tissue to *B. allii* is associated with the low nitrogen content of apple fruit, and that when inoculations are made in such a way as to make good this deficiency, definite attack takes place. This conclusion was amplified by the observation that *B. allii* produces a pectinase enzyme, capable of decomposing apple tissue, on a variety of media but not on apple extract, unless the latter is fortified by the addition of some nitrogenous compound.

The following is an account of a similar investigation, carried out with parasites of another pair of host plants, viz. apple and potato.

## II. MATERIAL AND METHODS.

The six fungi which have been used in this study are as follows :

1. *Botrytis cinerea*, Pers.
2. *Fusarium fructigenum*, Fries. Strain D of Brown.
3. *Fusarium fructigenum*, Fries. Strain B iii of Brown.
4. *Fusarium caeruleum* (Lib.), Sacc.
5. *Phytophthora erythroseptica*, Pethybr.
6. *Pythium* sp. (*de Baryanum*, Hesse?).

Nos. 1 and 2 are parasitic on apple fruit. No. 3 is a form which is very weakly parasitic on apple. Nos. 4-6 are parasitic on potato tubers.

In order to ensure purity each fungus was cultured from a single hyphal tip in the first instance. Stock cultures were kept in tubes of Brown's medium.

So far as host material is concerned, the investigation has been limited to large compact structures such as apple fruit, potato tubers, and to a certain extent turnip root tissue. The experimental work has involved the preparation of tissue extracts, and in some cases the setting up of sterile living host tissues. It is obvious that large solid organs such as those mentioned offer considerable conveniences from the experimental point of view.

The potato varieties used were 'King Edward' and 'Kerr's Pink'. Among apples, the variety 'Newton' was chiefly used because it is available in the market for rather an extended period.

*Method of Inoculation.* Inoculations were made by introducing the fungus into the host tissue so as to eliminate the effect of cuticle or cork

layer as an obstacle to infection. The plug method of Granger and Horne (3) was found to be most suitable. In order to secure the greatest degree of uniformity, a hard rubber band was placed round the cork borer so that the plug of tissue cut out was always of the same depth. After insertion of the inoculum, for which a standard spore suspension was used (except in the case of *Pythium* and *Phytophthora* where a piece of mycelium together with spores was used), the plug was reinserted and the wound sealed on the surface with wax. As a rule comparative inoculations were made on the same apple or potato in order to minimize the error due to variation of resistance from one individual to another. In control inoculations, sterile water replaced the spore suspension or piece of mycelium, but otherwise the treatment was the same. The experimental apples were wrapped in grease-proof paper which had been sterilized by exposure to chloroform vapour for two hours. The potatoes were not wrapped but were stored in large moist dishes.

The degree of attack was determined in each case by weighing the amount of tissue rotted in a given time. Frequent re-isolations of the fungi used were made, and in any case where contamination appeared, the results were discarded.

As regards the technique of enzyme extraction, details will be given later.

### III. EXPERIMENTAL RESULTS.

#### A. Observations on the Amount of Attack Produced when the Various Fungi were Tested on their Own Hosts.

The three fungi attacking apple fruit show a range of virulence, the most active being *B. cinerea*, *F. fructigenum*, Strain D, being next, and Strain Biii of the latter species least active, and in fact only able to attack occasional apples slightly. Of the potato-attacking fungi, *Pythium* sp. is the most virulent, causing the complete rotting of a good-sized tuber in a few days, *Phytophthora erythroseptica* is next, and *F. caeruleum* is the slowest.

Table I gives the average amounts of attack produced in a series of experiments in which the Strain D of *F. fructigenum* was compared with Strain Biii. Each test apple was inoculated with both fungi, so as to eliminate as far as possible the factor of varying resistance from apple to apple. The last column in the table gives the function  $t$  (Fisher, 2) from which the probability of the significance of the difference observed can be evaluated. With the numbers of apples used in each experiment, a value of  $t = 2.2$  is just significant, corresponding to a probability of 20:1, and for values of  $t$  greater than this, the degree of significance rapidly increases. It will thus be seen that the difference in amount of attack recorded in

each experiment is significant. This result confirms the earlier findings of Harvey (4) on this point.

TABLE I.

Exp.	Variety of apple.	No. of apples.	Average attack.		<i>t</i> .
			D. gram.	B iii. gram.	
1	Jonathan	12	1.9	0.0	5.0
2	"	12	2.7	0.2	2.2
3	"	10	6.2	0.0	3.3
4	"	10	3.2	0.8	3.5
5	Newtown	10	8.95	0.0	3.7

The greater parasitic activity of Strain D as compared with Strain B iii is also shown when tested on pear fruit (Table II). On each pear three wounds were made, one of these being inoculated with Strain D, another with Strain B iii, and the third left uninoculated. The figures indicate that pear tissue possesses less resistance, seeing that the weak Strain B iii causes an appreciable amount of rotting.

TABLE II.

Pear.	Attack (in 2 weeks) by		Uninoc. control.
	D. gram.	Biii. gram.	
1	31.4	12.0	Sound
2	39.0	13.9	"
3	44.1	6.2	"
4	31.2	2.1	"
5	40.4	7.8	"
6	35.6	2.9	"
7	37.4	6.5	"
8	31.2	7.5	"
9	48.6	18.7	"
Mean	35.4	8.6	

The *t* function for this comparison is 10.3 which represents an enormously high probability that Strain D is the more active fungus.

The resistance of potato tubers to attack by *F. caeruleum* and of apples (Newtown var.) to *F. fructigenum*, Strain D, was found to diminish with the state of ripeness of the host.

As regards potatoes (Kerr's Pink var.), tubers inoculated on November 1 and incubated at 20° for four weeks gave the following amount of rot:

Mean (for twelve potatoes) =  $2.52 \pm 0.16$  gram.

A similar experiment begun on December 4 with potatoes of the same batch gave as follows:

Mean (for twelve potatoes) =  $22.0 \pm 0.63$  gram.

The difference in amount of attack was thus 19.48 gram., a figure which

is twenty-seven times the probable error of the difference, and which is therefore highly significant.

With apples, experiments were carried out at different periods over most of the year. The variety 'Newtown' appears first in the shops in September, and it was possible to obtain fruit (cold-stored) up till the following August. The data of comparable infection tests are presented in Table III. The inoculated apples were in all cases maintained at 20° C., and each experiment was allowed to run for two weeks.

TABLE III.

Time of Year.	No. inoculated.	No. infected.	Average amount of attack. gram.
17 Oct.—2 Nov.	10	8	1.57 ± 0.29
25 Oct.—10 Nov.	12	10	2.0 ± 0.35
5—20 July	9	9	6.6 ± 1.3

The general tendency for a fall of resistance with normal ripening is clearly shown in the above table. A similar result was obtained, using apples which had been subjected to a process of artificial ripening. This process consists in incubating apples at 35° C. for one week, the controls being maintained during this time at ordinary temperatures (15° C.).

One experiment in which *F. fructigenum*, Strain D, was tested on twenty apples (ten normal, ten artificially ripened) gave the following results:

Average amount of attack on artificially ripened apples  
=  $5.84 \pm 0.53$  gram.

Average amount of attack on normal apples =  $1.57 \pm 0.35$  gram.

Experiments were also carried out in which half-apples were used so as to eliminate the effect of variable resistance from one apple to another. One half of each apple was wrapped up in grease-proof paper and subjected to the heating process, the other half being kept meanwhile at ordinary temperatures. The data are contained in Table IV.

TABLE IV.

Fungus	Half-apples.	Heated. gram.	Unheated. gram.	<i>z</i> .
Strain D	12	6.75	2.0	6.1
"	11	10.05	1.5	4.4
Strain B iii	9	0.25	0.0	5.5

It will be noticed that even the weak strain B iii is able to cause a slight but definite amount of attack on the artificially ripened apples.

Atmospheric humidity was shown to be a factor of importance in



determining the rate of attack of potato by *F. caeruleum*, but its influence on the rate of attack of apples by *F. fructigenum*, Strain D, appeared to be negligible. Experiments in this connexion were carried out at three temperatures as shown in Table V.

TABLE V.

Temp.	Condition of storage.	Average attack (2 weeks for apple, 4 weeks for potato)	
		<i>F. fruct.</i> on apple.	<i>F. caer.</i> on potato.
		gram.	gram.
15°	{ Dry	1.40	0.84
	{ Moist	2.10	3.75
20°	{ Dry	2.80	4.3
	{ Moist	2.76	20.6
25°	{ Dry	6.40	0.1
	{ Moist	6.00	0.5

*B. Behaviour of the Various Fungi when Tested on an Unsuitable Host.*

(a) *Apple-attacking fungi tested on potato.*

No attack of potato tubers by *B. cinerea* or *F. fructigenum* (Strain D) was obtained either at the beginning of the season, or towards the end when, as shown above, the host parts were more susceptible. Tubers of Kerr's Pink, King Edward, May Queen, and Jersey were all immune to attack.

According to Weiss, Lauritzen, and Brierley (6) the central tissue of the potato tuber is more susceptible than the peripheral parts owing to its reduced capacity for cork formation. The inoculation plugs were therefore cut so as to reach the centre of the tubers, and the latter were then stored at 10° C. so as to delay cork formation. Even under these presumably favourable conditions no attack was shown.

With both fungi it was observed that the spores of the inoculum germinated freely in the cavity, and in the case of *B. cinerea* conidiophores were even seen to develop. In a number of experiments the spores were sown in nutrient solutions—Brown's solution, Richards's solution, potato extract, and apple extract. Vigorous germination took place, but still no attack followed.

(b) *Potato-attacking fungi tested on apple.*

*Fusarium caeruleum*, *Pythium* sp., and *P. erythroseptica* all failed to attack apple fruit under normal conditions. The following varieties were tested with *F. caeruleum*: Newtown, Jonathan, Winesap, Five Crown, Worcester Pearmain, and Cox's Orange Pippin. In all cases the spores of the fungus were seen to germinate, but there was no attack.

Sowings of spores (*F. caeruleum*) were made on apple in potato

extract, apple extract, and Richards's solution. Germination took place in all cases, but only the sowings in Richards's solution produced appreciable attack. In order to analyse this effect, sowings in Richards's solution were compared with sowings in each constituent in turn of that solution. It was then found that the important constituent in this connexion was potassium nitrate. Illustrative results obtained from two experiments are given in Table VI.

TABLE VI.

Exp.	Spores sown in :	No. of apples.	Average amount of attack. gram.
1	Richards's solution	12	1.40
	Water	"	0.0
	1% $\text{KNO}_3$	"	1.00
	5% Cane sugar	"	0.0
	Richards's solution	"	0.63
2	Water	"	0.0
	0.5% $\text{KH}_2\text{PO}_4$	"	0.0
	0.25% $\text{MgSO}_4$	"	0.0
	Richards's solution	"	0.0

The stimulating effect on rate of attack produced by an added source of nitrogen is also shown when such fungi as *F. fructigenum*, Strain D, and *B. cinerea*, which normally attack apple, are used. The results of an experiment with twelve apples are set out in Table VII.

TABLE VII.

Fungus.	Average amount of attack when spores are sown in :		
	Water.	Richards's solution.	1% $\text{KNO}_3$
	gram.	gram.	gram.
<i>F. fruct.</i> (D)	0.85	2.05	2.2
<i>B. cinerea</i>	1.56	3.0	3.43

These results are in agreement with those of Vasudeva (5) concerning the relation between *B. allii* and apple tissue.

TABLE VIII.

Apple.	Amount of attack on :	
	Ripened half.	Unripe half.
	gram.	gram.
1	0.0	0.0
2	0.0	"
3	2.8	"
4	0.25	"
5	0.57	"
6	0.70	"
7	0.70	"
8	0.25	"
9	0.65	"
10	0.27	"
Average	0.62	0.0 $t = 2.3$

When apples which had been artificially ripened by the process mentioned above were inoculated in the ordinary way with spores of *F. caeruleum*, it was found that a slight amount of attack was shown. This result is illustrated in Table VIII in which a comparison is made between ten half-apples which have been put through the heat treatment and the corresponding halves which had meanwhile been stored at ordinary temperature.

Similarly it was found that when the apples were very ripe, e.g. in August for Newtowns, *F. caeruleum* was able to produce some attack when the spores were sown in water only, though here again the addition of a source of nitrogen to the inoculum increased the rate of attack. The average amounts of attack (twelve apples) obtained during August with apples of the previous year's crop were as follows:

Spores sown in water	1.05 grm.
" " " Richards's soln.	2.04 "
" " " 1 per. cent. $\text{KNO}_3$	3.06 "

The results of this section may be summarized in the following conclusions:

1. The apple-attacking fungi, viz. *B. cinerea* and *F. fructigenum*, Strain D, do not attack potato under any of the conditions tested.
2. The potato-attacking fungi, *F. caeruleum*, *P. erythroseptica*, and *Pythium* sp. do not attack apple fruit under normal conditions.
3. Spores of *F. caeruleum* are able to produce some attack on apple tissue when given an additional source of nitrogenous food, or when the apples are at an advanced stage of ripeness (whether arising naturally or produced artificially). Whether the same conclusions apply to *P. erythroseptica* and *Pythium* sp. was not determined.

#### C. *Study of the Enzymes Secreted by the Various Fungi.*

It has already been shown that the spores of all the fungi tested germinate freely when placed on the wounded surface of the plant tissue (apple or potato) whether the latter is susceptible or not. Failure to attack is therefore not due to any deleterious action of the plant juice, so far as germination of the spores is concerned. In looking round for a possible cause of the failure to produce attack, one naturally examines the enzymic behaviour of the different fungi, as there are strong grounds for the view that the active agent in parasitism of the type under consideration is the enzyme pectinase.

##### (a) *Method of preparation.*

The enzymic preparations (except for *P. erythroseptica* and *Pythium* sp.) were made by the method described by Brown (1) for *B. cinerea*.

The spores were sown on horizontal plates in a thin film of nutrient solution, and after forty-eight hours' growth the germ-tubes were collected, washed, and dried in vacuo. The dried fungal material was then ground with an equal weight of silver sand, giving a powder which could be kept for an indefinite time. The powder was extracted with water (0.2 gm. of powder to 3 c.c. water), and cleared by centrifugalizing, giving a solution which will be referred to as the *Standard enzyme extract*.

The nutrient solution used for germinating the spores was chiefly potato extract. This was prepared by squeezing the juice from slices of freshly peeled potatoes by means of a hand press, and freeing from suspended starch in the centrifuge. This crude juice was treated differently in different cases as will be shown later.

A modified technique was found to be necessary for the fungus *P. erythroseptica*. In this case it was impracticable to prepare spore suspensions in quantity. Liquid cultures in flasks were made and seeded with pieces of the fungal mycelium. In this case no enzyme could be demonstrated either in the mycelial mat when ground to powder or in the liquid in which the fungus had grown. Nevertheless, it seemed obvious, from the type of soft rot produced, that the fungus produces the enzyme in quantity. The following method of preparation was finally adopted.

Large-sized potato tubers were thoroughly washed, peeled, and then sterilized on the surface with mercuric chloride (1/1,000).<sup>1</sup> After washing in sterile water they were placed in sterilized jam-jars containing moist cotton wool and inoculated with *P. erythroseptica*. After about six days' incubation at 20° a large portion of the tuber had become infected. The rotted portion was then extracted under slight pressure, and the crude extract so obtained was used forthwith for tests of enzymic action.

A similar method was used for preparing solutions of the enzyme of *Pythium* sp.

The activity of the various enzymic preparations was determined by noting the time required under standard conditions for the disintegration of potato, turnip, or apple discs of standard (50  $\mu$ ) thickness. The time taken for disintegration is in inverse ratio to the activity of the preparation.

(b) *Factors influencing the activity of the pectinase enzyme of B. cinerea.*

The failure of *B. cinerea* to attack potato tissue might be explained on the grounds that the fungus produces an alkaline reaction when grown in potato juice and that the degree of alkalinity so produced inhibits the functioning of the enzyme. To test this view, determinations were made of the relation between H-ion concentration and enzymic activity. The standard enzyme extract is faintly acid and a range of H-ion concentrations

<sup>1</sup> It was found to be impossible to sterilize unpeeled tubers, probably because bacteria are lodged in the lenticels. Such tubers always became infected with bacterial growth later on.

towards the alkaline side was made up by adding drops of N/10 caustic soda. The pH's of these preparations were determined by the colorimetric method. Test discs were then inserted and the times required

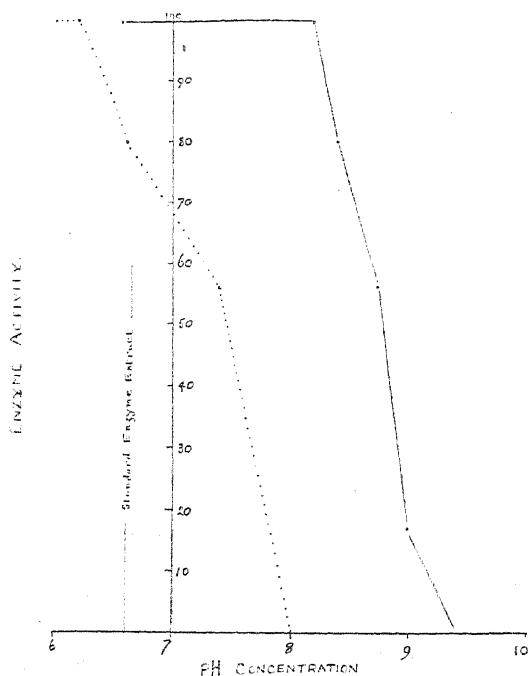


FIG. 1. Illustrating relation between enzymic activity and pH concentration.

for disintegration noted. At the end of the experiment the pH's were again determined and found to have shifted appreciably towards the acid side. This effect is presumably due to the escape of acid cell-sap from the test discs. Fig. 1 shows the relation between enzymic activity and pH concentration, the heavy line corresponding to *initial* pH, and the dotted line to final pH. It is clear that the true graph would lie somewhere between the two.

It should be added that a slight amount of dilution takes place as a result of adding drops of decinormal alkali. This dilution itself is of no significance, as was shown by a subsidiary experiment.

These results are in accordance with those obtained by Brown (l.c.), although in this case the fall in activity on the alkaline side is not so sharp. It is possible that the precise result obtained may vary somewhat from one strain of the fungus to another, or with the method of preparing the enzyme.

While it is shown in the above figure that enzymic activity disappears at a pH which lies between 8.0 and 9.4, other results do not appear to

agree. Thus when spores are sown on plates in potato juice the reaction of the latter was found after two days to be in the neighbourhood of  $\text{pH} = 9.6$ , and yet that liquid contained active enzyme. There is some discrepancy, perhaps arising from the methods used.

The subject was not fully explored, as it appeared from other experiments that the development of alkalinity was probably not the factor responsible for failure to attack. When spores of *B. cinerea* are sown in drops of Richards's solution on potato tissue, no attack follows, even though the nutrient used is distinctly acid and is only slowly converted to alkaline by the growth of the fungus. Moreover, care was taken to maintain an acid reaction in the inoculum cavity by adding further drops of Richards's solution from time to time. In spite of this maintained acidity attack did not take place.

Experiments were carried out to determine whether certain plant juices (potato, apple, turnip) contained any substances which interfered with the activity of *Botrytis* enzyme. A standard enzyme extract was made up and a series of dilutions prepared, using as diluting liquid: (a) water; (b) plant juice; and the enzymic activities of the various dilutions were determined in the usual way. Table IX gives the result of such a comparison, the figures being the times (in hours) for disintegration of standard potato discs.

TABLE IX.

Concentration of enzyme.	Diluting liquid.			
	Water.	Potato extract.	Apple extract.	Turnip extract.
	hr.	hr.	hr.	hr.
100% (= undiluted)	1	1	1	1
80%	1	3	1.8	1
60%	1	6	2.3	1.3
50%	1	22	3.8	1.8
40%	1.25	33	4.25	1.8
30%	1.5	>48	6	2.5
20%	2.5	>48	6	2.8
10%	2.5	>48	10	3.5

The second column gives the effect on activity due to dilution only. When potato extract is used as diluting liquid enzymic activity falls off much more rapidly, and at a concentration somewhere between thirty and forty per cent. of standard strength activity disappears altogether, or at any rate is so slight that the test discs appear totally unaffected after two days' treatment. The effect of turnip extract is not markedly different from that of pure water. Apple extract has a greater retarding action than turnip extract, but its action in this respect is much less than that of potato extract.

It thus appears that the enzyme of *B. cinerea* is particularly sensitive to the presence of potato juice. In the first experiments in this connexion crude unsterilized potato juice was used. It was found, however, that the same effect was obtained with boiled or autoclaved juice. Again, a sample of potato juice was evaporated to dryness, the residue calcined, and the ash taken up in water to the original volume. The solution of ash constituents was found to possess a retarding action on the enzyme of *B. cinerea* quite comparable to that of fresh potato extract.

In order to analyse this effect more fully, tests were made with Brown's solution of five times the usual strength, the composition of which is comparable to that of undiluted potato extract, at least as regards the more important constituents. This synthetic solution<sup>1</sup> gave a strong retarding action on *Botrytis* enzyme, and when the effect of each constituent in turn was tested, it was found that the two important elements in this connexion were the magnesium sulphate and potassium phosphate. Figures illustrating these results are given in Table X.

TABLE X.

Diluting liquid.	Concentration of enzyme extract.		
	100 %.	50 %.	30 %.
	hr.	hr.	hr.
Water . . . . .	1.5	2.5	3.5
Complete synthetic . . . . .	"	> 48	> 48
Starch 5 % . . . . .	"	2.5	3.5
Glucose 1 % . . . . .	"	2.5	3.5
Asparagin 1 % . . . . .	"	2.5	3.5
K <sub>3</sub> PO <sub>4</sub> 0.6 % . . . . .	"	24	> 48
MgSO <sub>4</sub> 0.4 % . . . . .	"	24	> 48
<sup>2</sup> Starch + Glucose + Asparagin . . . . .	"	2.5	3.5
<sup>2</sup> K <sub>3</sub> PO <sub>4</sub> + MgSO <sub>4</sub> . . . . .	"	> 48	> 48

The retarding action of potato juice on the activity of *Botrytis* enzyme is therefore fully explained on the basis of its content of magnesium sulphate and potassium phosphate.

Further tests showed that a solution of magnesium chloride (0.4 per cent.) has a retarding action quite similar to that of the same concentration of magnesium sulphate, whereas the action shown by a solution of ammonium sulphate (0.4 per cent.) is slight. These results therefore indicate that the retarding action of magnesium sulphate is mainly to be ascribed to the magnesium radical. The further analysis of the retarding action of potassium phosphate has not been carried out.

<sup>1</sup> Glucose 1 per cent., asparagin 1 per cent., K<sub>3</sub>PO<sub>4</sub> 0.6 per cent., MgSO<sub>4</sub> 0.4 per cent., and starch 5 per cent.

<sup>2</sup> Same concentration as above.

(c) *Comparative study of pectinase enzymes of various fungi.*

Experiments similar to those illustrated in Table IX were carried out with the enzymic preparations derived from *F. fructigenum* Strain D, *F. caeruleum*, *P. erythroseptica*, and *Pythium* sp. The results are summarized in Table XI, the figures giving, as before, the time required for disintegration of standard potato discs. The two *Fusarium* species gave preparations so weak that it was considered advisable to keep them in an atmosphere of chloroform vapour during the test period in order to prevent bacterial contamination.

TABLE XI.

Fungus.	Diluting liquid.	Concentration of enzymic extract.			
		100 %.	50 %.	30 %.	10 %.
		hr.	hr.	hr.	hr.
<i>F. fruct.</i> (D)	{ Water	24	36	> 48	> 48
	{ Potato juice	"	> 48	"	"
	{ Apple juice	"	36	"	"
<i>F. caeruleum</i>	{ Water	24	> 48	"	"
	{ Potato juice	"	24	"	"
	{ Apple juice	"	> 48	"	"
<i>Phyt. eryth.</i>	{ Water	1.5	1.5	1.5	2
	{ Potato juice	"	1.5	1.5	2.5
	{ Apple juice	"	7.5	> 48	> 48
<i>Pythium</i> sp.	{ Water	0.5	0.5	1.0	1.5
	{ Potato juice	"	0.5	1.0	2.0
	{ Apple juice	"	0.5	2.0	> 48

With the active preparations obtained from *Phytophthora* and *Pythium* it is clear that apple juice has a retarding action much greater than has potato juice, the latter not differing appreciably from pure water. The two species of *Fusarium* gave enzymic preparations so weak that fewer significant measurements of activity could be obtained, but as far as the results go they indicate that the enzyme of *F. fructigenum* is more retarded by potato juice than by apple juice while the converse holds for the enzyme of *F. caeruleum*.

Experiments were carried out to determine what constituent of apple juice is chiefly responsible for reducing the activity of *Pythium* enzyme. In contrast with the results for potato juice described above, the retarding action of apple juice was not reproduced by the ash constituents. Thus the active ingredient was not a mineral salt.

In order to carry out the analysis of this effect a synthetic solution was prepared which conformed approximately to the average composition of apple juice.<sup>1</sup> The composition of this solution was as follows:

Glucose	18 grm.
Cane sugar	6.5 "

<sup>1</sup> Data obtained from Thorpe's Dictionary of Chemistry Art. 'Apple'.



Asparagin	0.12 grm.
KH <sub>2</sub> PO <sub>4</sub>	0.12 „
MgSO <sub>4</sub>	0.01 „
KCl	0.36 „
Malic acid	1.11 „
Water	200 c.c.

The effect of this solution in retarding the action of *Pythium* enzyme was found to be comparable to that of apple juice, and by adopting the same method of analysis as illustrated in Table X above, it was found that the retarding action of apple juice was mainly due to its content of malic acid. The data obtained are shown in Table XII.

TABLE XII.

Diluting liquid.	Standard <i>Pythium</i> enzymic extract concentration.			
	100 %.	50 %.	30 %.	10 %.
	hr.	hr.	hr.	hr.
Water . . . . .	0.8	1.0	1.3	2.8
Apple juice . . . . .	„	1.8	4	>48
Complete synthetic solution . . . . .	„	2.8	3.8	>48
Glucose constituent . . . . .	„	1.0	1.3	2.8
Cane sugar constituent . . . . .	„	1.0	1.8	2.8
Asparagin „ . . . . .	„	1.0	1.3	2.8
KH <sub>2</sub> PO <sub>4</sub> „ . . . . .	„	1.0	1.3	2.8
MgSO <sub>4</sub> „ . . . . .	„	1.0	1.3	2.8
KCl „ . . . . .	„	1.0	2.8	4.3
Malic acid „ . . . . .	„	1.8	3.8	>48
Neutralized apple juice . . . . .	„	1.0	1.3	2.8

The pectinase enzyme of *Pythium* is thus much more sensitive to acidity than is the corresponding enzyme of *B. cinerea*. On the other hand, it appears to have a much greater tolerance to alkali, its greatest activity being manifested at a distinctly alkaline reaction. Comparable data for the two enzymes are given in Table XIII, the maximum activity of each being taken as 100.

TABLE XIII.

pH	Activity of <i>Pythium</i> enzyme ; <i>Botrytis</i> enzyme.	
8.0	100	0
5.5	42	100
5.0	20	100

It is noteworthy that the differential behaviour of the enzymes of these two fungi just described runs parallel to the differential growth shown by the fungi themselves on media of varied acidity or alkalinity. Fig. 2 gives the rate of growth of *Pythium* and *Botrytis* cultures on potato agar

plates which have been adjusted to different pH's by addition of the requisite amounts of malic acid or sodium bicarbonate. Whether the same relationships hold as regards *P. erythrosepica* has not been determined

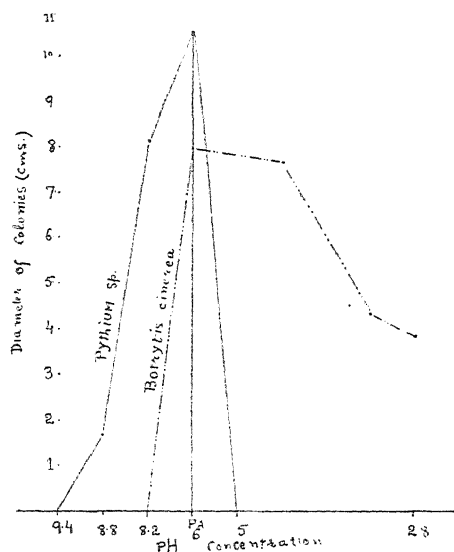


FIG. 2. Illustrating growth of *Pythium* sp. and *B. cinerea* on potato agar media over a range of pH concentrations.

(d) 'Hardening' effect on cell-wall tissue of certain plant extracts.

Brown (l.c.) has stated that when potato discs are placed for some time in certain plant extracts (e.g. of bean leaves) they become less sensitive to the dissolving action of *Botrytis* enzyme. This result has been confirmed in the present work. Thus when living discs of potato are kept in water overnight and then tested with *Botrytis* enzyme, they are decomposed in the same time as discs freshly cut from the same potato. When, however, they are left for the same time in potato extract, their sensitiveness is much diminished, and they require in fact a treatment three times longer to effect disintegration. Turnip discs kept in potato juice are similarly 'hardened'. On the other hand, turnip juice has no similar action on turnip or potato tissue. Apple juice has an intermediate behaviour. Data relative to this point are contained in Table XIV.

When crude unboiled potato juice is used there is a certain amount of darkening of the liquid and of the surface of the plant tissue on account of oxidase reactions. That these reactions are not concerned in the 'hardening' process is shown by the fact that the same results are obtained when the preparations are stored in vacuo (so that no oxidation can take place) and also when the juice is boiled, thereby destroying the oxidase enzyme

TABLE XIV.

Treatment.	Disintegration time.	
	Potato discs.	Turnip discs.
	hr.	hr.
Freshly cut . . . . .	0.8	1
Soaked for 20 hr. in water . . . . .	0.5-0.8	1
"    "    Potato juice . . . . .	4	4
"    "    Turnip " . . . . .	0.8	1.3
"    "    Apple " . . . . .	2	2

present in it. Furthermore, the action of potato juice in rendering tissue less sensitive to the action of *Botrytis* enzyme can be imitated by using the synthetic extract mentioned on p. 1044. Further analysis has shown that the active constituents in this connexion are magnesium sulphate and potassium phosphate. Data on this point are contained in Table XV.

TABLE XV.

Treatment.	Disintegration time.	
	Potato discs.	Turnip discs.
	hr.	hr.
Freshly cut . . . . .	1.5-2	1.5
Soaked for 20 hr. in water . . . . .	1.5-2	1.5
"    "    "    Potato juice . . . . .	7-8	—
"    "    "    Synthetic solution . . . . .	7-8	—
"    "    "    K <sub>2</sub> PO <sub>4</sub> " . . . . .	7-8	8
"    "    "    MgSO <sub>4</sub> " . . . . .	7-8	3.5

#### IV. DISCUSSION OF RESULTS.

The chief interest of the present paper lies in the enzymatic relationships suggested.

So far the behaviour of three fungi parasitic on potato and of two parasitic on apple has been examined in more or less of detail. The fungi attacking potato do not, except in special circumstances, attack apple, and conversely for those attacking the latter host.

Preparations of the pectinase enzyme have been made from all five fungi. Those from *B. cinerea*, *Pythium* sp., and *P. erythroseptica* proved to be very active when tested either on potato or apple tissue. The preparations from the *Fusarium* species were very weak, but so far as tests could be made with them, the enzyme of *F. fructigenum* showed a similar behaviour to that of *B. cinerea*, while the enzymatic preparation of *F. caeruleum* was similar to that of *Pythium* or *P. erythroseptica*.

The pectinase preparations of *B. cinerea* differs from those of *Pythium* and *P. erythroseptica* in being much more strongly retarded by given concentrations of potato extract and in having its optimum for activity in acid solution. The preparations of the latter two fungi, on the other hand, are

more retarded by additions of apple than of potato extract and are most active in alkaline solution. Some kind of specificity is thus indicated.

It seems highly unlikely that the fungi characteristic of any one host should possess a pectinase enzyme specifically different from that of the fungi of any other host. The greater likelihood is that it may be possible to distinguish a limited number of types of pectinase. An alternative possibility is that the enzyme may be the same in all cases, but that the details of its behaviour may be modified by the presence of other substances.<sup>1</sup> Further investigation may thus lead to the study of host constituents or of other metabolic substances of the fungus as influencing the reactions of the pectinase enzyme.

This work was carried out at the suggestion and under the direction of Professor W. Brown, to whom I wish here to express my thanks.

#### V. SUMMARY.

1. *F. fructigenum* Strain D, and *B. cinerea* which are parasitic on apple tissue are unable to attack potato tissue; similarly *F. caeruleum*, *P. erythroseptica*, and *Pythium* sp. which are parasitic on potato do not under normal conditions attack apple tissue.

2. A certain amount of invasion of apple tissue by *F. caeruleum* was shown when the inoculum of the latter was supplied with an additional source of nitrogenous food. Increased virulence was also shown by *B. cinerea* and *F. fructigenum* Strain D, under the same conditions.

3. The resistance of apple to attack by *F. fructigenum* Strain D, and of potatoes to *F. caeruleum* diminishes as the host tissue becomes riper. When apple fruit is fully ripe it becomes susceptible to attack even by *F. caeruleum* and by a very weak Strain (Biii) of *F. fructigenum*.

4. The failure of the fungi normally parasitizing the one host to attack the other is not due to any failure of the juice of the latter to effect germination of the fungal spores.

5. It has been found that the pectinase enzymes of the two fungi attacking apple are more readily deactivated by potato juice than are enzymes prepared from the three potato-attacking fungi. The active principle in this connexion is the mineral content of potato juice, more particularly as regards magnesium and potassium phosphate.

6. The enzymes of the three potato-attacking fungi are comparatively insensitive to the action of potato juice, but are sensitive to the presence of apple juice. The factor responsible in this case is the concentration of malic acid. The enzyme of *Pythium* sp. has been found to have its greatest activity on the alkaline side of neutrality, and to be very sensitive to an acid reaction; whereas the converse behaviour is shown by the

<sup>1</sup> Work in progress indicates that this is the probable interpretation.

enzyme of *B. cinerea*. It is pointed out that the growth curves of these two fungi show the same features in relation to pH concentration.

7. Potato juice, in addition to its retarding effect on the enzyme of *B. cinerea*, also renders certain plant tissues (potato, turnip) less sensitive to the action of *Botrytis* enzyme. This effect also is due to the mineral salt constituents of potato juice.

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# A Self-recording Potometer with a Note on Rate of Transpiration under Pressure.<sup>1</sup>

BY

F. M. HAINES, PH.D.

With six Figures in the Text.

THE mechanism to be described was devised to meet the necessity for a more sensitive self-recording potometer than those devised hitherto for use in connexion with an apparatus for the comparison of transpiration rates of plants under pressure, but is capable of a wider application.

The general principle is that of a Ganong potometer in which the uptake of water is allowed to move a column of B.P. paraffin which, by means of an automatic electrically operated slide-valve, causes a small mercury column to move backwards and forwards between two contacts in a capillary tube, an electric pen being actuated every time the mercury makes one of the contacts. This contact is made after the absorption of equal small volumes of water or the movement of equal small volumes of paraffin, which may be made at will as small as 0.002—0.0001 c.c.

The apparatus consists in a potometer vessel, A (Fig. 1), in which the plant is placed with the usual precautions. This vessel is connected by means of rubber connexions and the glass tube, B, with a vessel, C, corresponding with the water reservoir in the Ganong potometer, and having the same function. The tube, DF, from the 3-way tap, D, below the container, C, instead of connecting directly with a capillary tube, connects only with the graduated capillary tube, L, through a 3-way tap, F, by which it can also be connected with the vessel, GMH, and (through the outlet, K), with the recorder, the capillary tube, L, only serving for calibrating purposes. The components, A, B, C, D, E, F, L (when in use), and G up to the level marked X, are filled with water supplied from the container, C. The upper part of the vessel, G, the 3-way tap, M, and the tube, K, are filled with liquid paraffin (B.P.) supplied from the container, H. The whole vessel, FGMHK, is necessary as paraffin has to be used instead of water in the recorder and the position of the water-paraffin

<sup>1</sup> From the Botanical Department, East London College.

meniscus, which must necessarily be in a vertical tube, needs to be under easy control. The level, X, moves down during experiments, and needs to be returned to the top of G, for which reason the container, C, is made

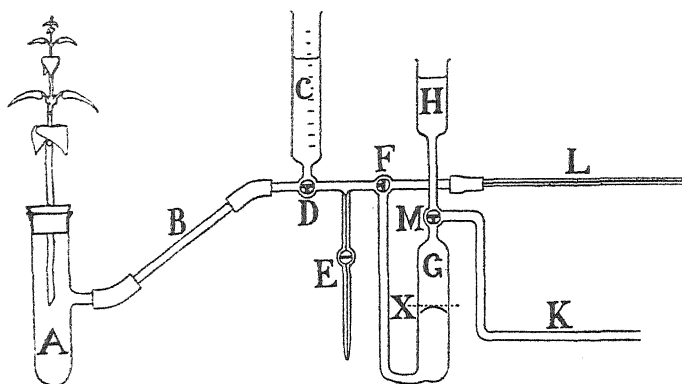


FIG. 1.

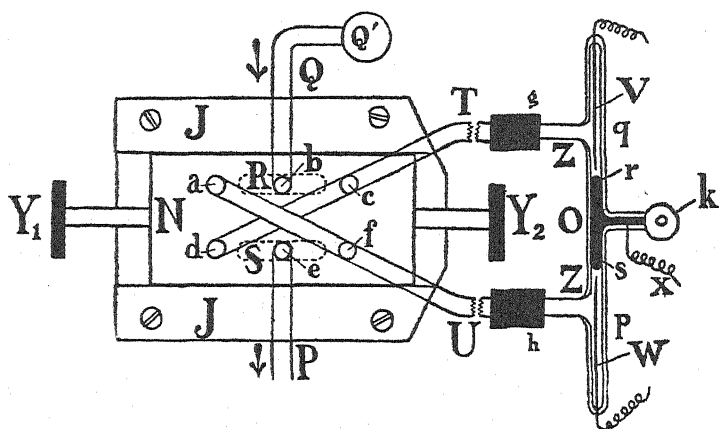


FIG. 2.

higher than H. The volume of G is about 20 c.c. The capillary tube and capillary tap, E, are used in calibration (see below), and are also convenient for drawing off water at the beginning of direct-reading experiments in order to bring the meniscus in the capillary tube, L, rapidly up to the zero.

The recorder consists essentially in a special form of slide valve, JJ (Fig. 2), and a glass capillary tube, ZZ, containing a short column of mercury, *rs*. The mercury column is continuously in connexion with a platinum wire contact, *x*, sealed through the middle of the tube, and moves backwards and forwards between the two platinum contacts, V and W, sealed through the ends of the tube, making contact with them alternately.

The tube, K, of the potometer (Fig. 1) is connected with the outlet, P,

of the slide-valve, and suction from the potometer causes the mercury column, *rs*, to be drawn towards one of the platinum contacts. The

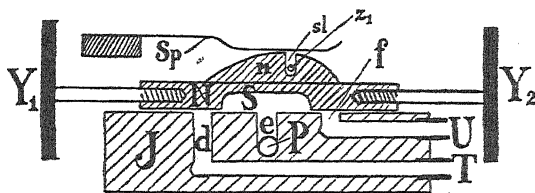


FIG. 3.

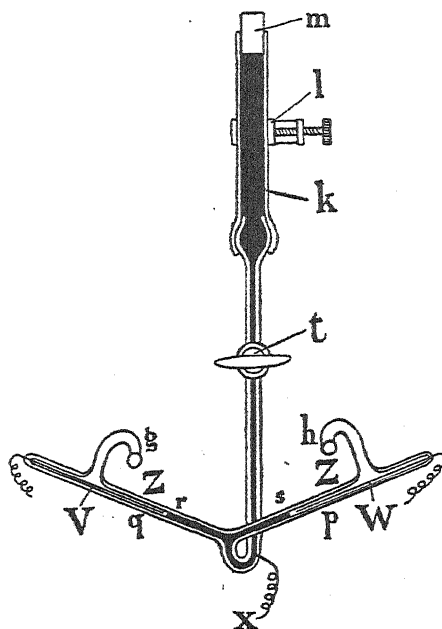


FIG. 4.

establishment of electrical connexion by the mercury column between *x* and *p* or *x* and *q* actuates relays which in turn actuate electro-magnets which move the moving part, *N*, of the slide-valve in such a way that every time the mercury reaches one of the contacts the valve reverses the direction of flow in the capillary tube, and causes the mercury to travel back again towards the other contact. Thus, when the mercury column at *s* touches the contact, *W* (Fig. 2), it closes the 2-volt circuit through the relay, *R*<sub>1</sub> (Fig. 5), which closes the break, *k*<sub>1</sub>, and thus actuates the electromagnet, *C*<sub>1</sub>. This attracts the soft iron armature, *Y*<sub>1</sub>, connected with the moving part, *N*, of the slide-valve, and brings the ports, *R*, *S*, in the slide (dotted in the diagram, Fig. 2), into such a position as to connect *P* with *T* and *Q* with *U*. The suction from the potometer is now therefore applied to the end, *r*, of



the mercury column causing it to move towards the contact, V. On establishment of contact between  $x$  and V (Fig. 5), the relay,  $R_3$ , is actuated, closing the break,  $k_2$ , which actuates the electro-magnet,  $C_2$ . This attracts the armature,  $Y_2$ , on the moving part of the slide-valve, and brings the ports, R, S, into such a position that they connect P with U and Q with T. Thus, while paraffin is drawn continuously to P from the reservoir, Q', connected with Q, as indicated by the arrows (Fig. 2), the direction of the suction on the mercury column is repeatedly reversed.

The slide-valve consists in a solid brass block, JJ, down into the flat upper surface of which are drilled the holes (2 mm. in diameter)  $a, b, c, d, e, f$ . Of these  $b$  connects with a hole drilled horizontally into the side of the block, and carrying an inlet tube, Q, which leads to the reservoir of paraffin, Q', while  $e$  connects with a horizontal hole drilled into the opposite side of the block, and carrying an outlet tube, P, which is connected with the tube, K, of the potometer (Fig. 1). The ports,  $a$  and  $f$ , are connected together by means of a horizontal hole drilled diagonally through the block near its upper surface and connecting with the brass tube, U, while  $c$  and  $d$  are connected together by a horizontal hole drilled diagonally through the block near its lower surface, and connecting with the brass tube, T (Fig. 3). It will be realized that U,  $a$ , and  $f$  are connected by the channels in the block and T,  $d$ , and  $c$  are connected, but that there is *no* connexion between  $a$  and  $d$  or between  $c$  and  $f$ . The two diagonal channels do not intersect, but cross at different levels (see Fig. 3). The sliding piece of the valve, N, consists in a flat brass slide moving between V-shaped guides on the upper surface of the block, and having on its under surface the two oblong ports, R and S (dotted in Fig. 2) (see Figs. 2 and 3), of which, in one position of the valve, R connects  $b$  and  $c$ , while S connects  $e$  and  $f$ , and in the other position R connects  $b$  and  $a$ , while S connects  $e$  and  $d$ . While the slide is in the left-hand position the flow of paraffin from Q to P is in the direction Q,  $b, a, U, p, q, T, nd, e, P$ , and when in the right-hand position, Q,  $b, c, T, q, p, U, f, e, P$ . In the first case the mercury column is drawn towards the contact, V, and in the second towards the contact, W. The slide moves between stops and is moved by the 240-volt electro-magnets,  $C_1, C_2$  (Fig. 5), acting upon the soft iron armatures,  $Y_1, Y_2$ , attached to the sliding piece. The mechanical finish of the slide-valve is of extreme importance. The working surfaces are pressed together by means of a strip of spring steel,  $Sp$  (Fig. 3), pressing downwards on a brass piece,  $n$  (Fig. 3), mounted on the slider, N. After the ordinary machining and grinding with fine carborundum powder the surfaces were thoroughly worked together and ground to a perfect fit by allowing the electric mechanism operating the valve (described below) to cause the valve to oscillate rapidly backwards and forwards for some hours, while the sliding surfaces were fed with a fine suspension of rouge in oil forced into the ports through P, Q, T, U. The

process was then repeated for a considerable time, using oil only. After thorough running in in this way there is no leakage of air or paraffin at the sliding surfaces, even with pressures approaching an atmosphere (with about 100 grm. weight tension in the spring), and the pressures which it is required to hold in actual use are of the order of 2-5 mm. of mercury. It will be noticed, moreover, that the paraffin in the valve is at less than atmospheric pressure, which would still further reduce any tendency to leakage if present. After running in in the way described the behaviour of the valve is perfectly satisfactory as checked by the complete absence of leakage under continuous pressure, using a column of paraffin in a capillary tube as indicator. The block measures 2.5 by 3.5 cm.

*Adjustment of the sensitivity.* The volume of paraffin which must be drawn up between successive reversals, and therefore the sensitivity of the recorder, depends only upon the difference between the length of the mercury column,  $rs$ , and the distance between the points of the platinum contacts, V and W (Fig. 4). This, and therefore the sensitivity, may be adjusted at will by altering the effective length of the mercury column. This is accomplished by the device shown in Fig. 4, which shows an elevation of the glass part bearing the tube, ZZ, in which the mercury moves. The centre of the tube, ZZ, is connected through a capillary tube and capillary tap,  $t$ , with a small reservoir of mercury, consisting in a short length of pressure tubing,  $k$ , plugged at the top by a short piece of glass rod,  $m$ , and carrying a screw clip,  $l$  (Fig. 4). By opening the tap,  $t$ , and slightly screwing up or releasing the clip,  $l$ , the length of the mercury column,  $rs$ , in the tube, ZZ, can be adjusted to a nicety, and the tap then turned off to ensure its remaining constant. The tube, ZZ, rises slightly at an angle of about  $20^{\circ}$ - $30^{\circ}$  on each side of its mid-point, in order to avoid the tendency which the mercury column shows in a perfectly straight horizontal tube to elongate slightly by flattening out at the ends. If, on the other hand, ZZ is made in the form of a U-tube, an unnecessarily large pressure difference is required to cause a contact, and this entails a loss of sensitivity. With the form of tube described the pressure difference required to work the recorder is practically only that necessary to overcome the viscosity of the paraffin, and amounts only to a few mm. of mercury.

It could probably be still further reduced if necessary by using lighter oils, but is of no consequence, since any small pressure difference which may be necessary to work the recorder can be exactly compensated for by raising or lowering A relative to K (Fig. 1), (hence the two rubber connexions to B), and need not necessitate the pressure in A at which absorption is carried on being anything other than exactly atmospheric. A manometer may be intercalated between A and D to facilitate the determination of the correct relative levels of A and K if desired, or the correct slant for B may be determined by replacing A momentarily by

a small nozzle, and finding the angle of B at which the tendency of the water to run down in B will *just* work the recorder.

The outlet tubes,  $g$ ,  $h$ , are made to lead out of ZZ on the upper side and later to curve over inwards and forwards (i.e. away from the plane of the paper in Fig. 4). This shaping is important, as it prevents the possibility of the mercury being carried over into the slide-valve in the event of a tap being turned in error, or the potometer being connected in such a way as to apply pressure or suction to the mercury when the electric circuits are not switched on; so that it does not become reversed on reaching the normal end of its travel.

If ZZ is made of tubing of 1 mm. bore, and the length of the mercury column is adjusted to 1 mm. less than the distance between the contacts, the mechanism is actuated by the absorption of less than 0.001 c.c. since the last movement. Every other movement is caused to actuate the electric pen, P' (Fig. 5), placed in parallel with the coil,  $C_1$ , and a mark on the recording drum is therefore made after every complete cycle of operations, i.e. after the absorption of each 0.002 c.c. By constructing ZZ of narrower capillary (e.g. 0.5 mm. diameter), or lengthening the mercury column, so that it only moves through 0.25 mm., the apparatus can be made to record every 0.0001 c.c. Down to this degree of sensitivity it is not sensitive to vibration.

The electric circuit and connexions are shown in detail in Fig. 5. The important feature of the circuit is the special device employed to eliminate sparking at the relay contacts,  $k_1$ ,  $k_2$  (Fig. 5), and the mercury contacts,  $rV$ ,  $sW$ , in the capillary tube. This is particularly important in the case of the mercury contacts, as any trace of arcing when the contact was broken would foul the contacts, and lead to uncertainty of action, as well as vaporizing some of the mercury and reducing the effective length of the column. The device which completely overcomes these difficulties and renders the make and break of the mercury contacts absolutely reliable is the automatic cut-out,  $x_1x_2$ , shown in the centre of the figure (Fig. 5). This is actuated by the moving part of the slide-valve, and every time a contact is made, either in the 2-volt circuit by the mercury or in the 240-volt circuit at  $k_1$  or  $k_2$ , automatically breaks the circuit immediately elsewhere, so that no current is passing when the mercury contacts or  $k_1$ ,  $k_2$  are broken. The cut-out consists in an arm of steel wire,  $z_1z_2z_3$ , working about a vertical pivot at  $y$ , and coiled once at  $z_2$  to cause a lag of the end  $z_3$  after movement of the actuated end,  $z_1$ . The end,  $z_1$ , passes through a slot,  $sl$ , in the brass piece,  $n$ , mounted on the top of the moving part of the slide-valve (Fig. 3). When the end,  $s$ , of the mercury column makes contact with W actuating  $R_1$ ,  $k_1$  is made, and  $C_1$  actuated so that the valve travels to the left. The end  $z_1$  of the cut-out arm is drawn to the left, and  $z_3$  therefore travels to the right. The two contact plates,  $x_1$ ,  $x_2$ , carried

on  $z_3$ , thereupon make contact respectively with the contacts,  $h_2$  and  $l_2$ . The plates  $x_1$  and  $x_2$  are insulated from one another,  $x_1$  being in connexion through the pivot,  $y$ , with the 240-volt negative terminal,  $H_-$ , and  $x_2$  being

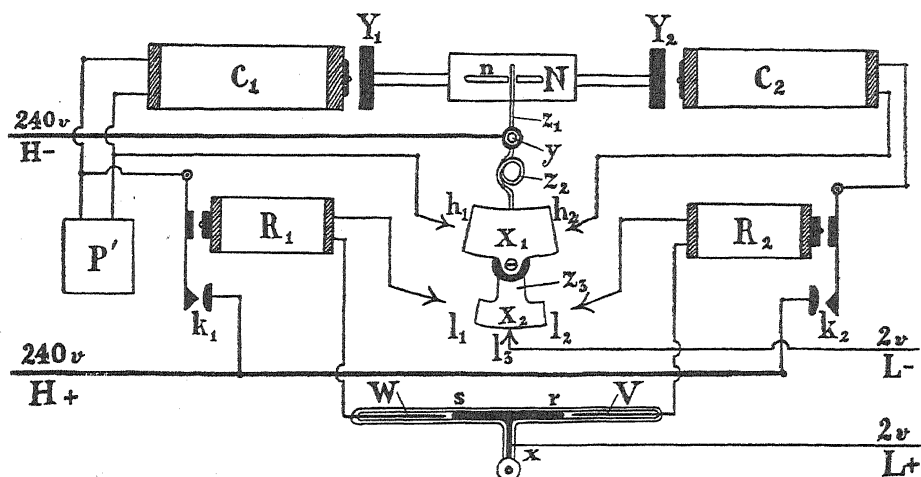


FIG. 5.

in continuous connexion with the contact,  $l_3$ , leading to the 2-volt negative terminal,  $L_-$ . The movement of the cut-out, therefore, makes it possible for  $C_2$  to be actuated next (when  $r$  makes contact with  $V$  and causes a make at  $k_2$ ), at the same time breaking both the relay and main circuits on the side of  $C_1$ . No spark can therefore ensue when the mercury surface at  $s$  leaves  $W$  nor at the relay contact,  $k_1$ . When  $r$  makes contact with  $V$  actuating  $R_2$ ,  $k_2$  is made, actuating  $C_2$  and the slide-valve moves to the right. This carries  $z_1$  to the right, causing  $x_1$  and  $x_2$  to move to the left, breaking both the circuits on the side of  $C_2$  and making  $h_1$  and  $l_1$  ready for the next actuation of  $C_1$ . All the contacts,  $h_1$ ,  $h_2$ ,  $l_1$ ,  $l_2$ , are made of thin sheet brass brushes of a similar shape to those in an ordinary tumbler switch though larger, the breaks in the high voltage circuit being about 2 cm., and in the low voltage circuit about 1 cm. The arm,  $z_3$ , works between stops (not shown) in order to prevent jamming in the brush contacts.

The coils,  $C_1$ ,  $C_2$ , and the coil of the electric pen,  $P'$ , each measure 5.5 cm. long and 2.5 cm. in diameter, and each carry approximately 3,000 turns of silk-covered 44-gauge copper wire. The relay coils measure 1.7 cm. long and 1 cm. diameter, and carry approximately 250 turns of silk-covered 26-gauge wire.

For the purpose of running in the valve it is only necessary to run sufficient mercury into  $ZZ$  (Fig. 4) from  $k$  to make contact at both ends,  $r$  and  $s$ , at once. This causes both  $k_1$  and  $k_2$  (Fig. 5), to be made

simultaneously, and N oscillates rapidly backwards and forwards like an electric bell armature until the mercury is withdrawn.

The whole apparatus, excluding the glass parts shown in Fig. 1, fits on to a base measuring 35 cm. by 14 cm.

The recorder is obviously equally applicable as a porometer, the tube, P, being connected with a falling tube of any desired length to create the suction, and Q being connected with the lower end of a reservoir of paraffin, the upper end of which is connected to the leaf chamber and a manometer for measuring the effective pressure at the leaf surface.

As a general rule, as in the experiments quoted below, only relative results are required, but the apparatus is easily calibrated for any given setting of the mercury column by shutting off the tap, D (Fig. 1), and allowing water to drop at a low constant rate from the fine nozzle at the end of the capillary tube, E. The rate is then read alternately by timing the rate of flow of the meniscus in the capillary tube, L, of known cross-section by means of a stop-watch and with the recorder. The switching over from one method of reading to the other is easily effected by use of the 3-way tap, F. In this way, absolute readings are easily obtained. In the records appended below the individual divisions correspond each to 0.001 c.c., and, the speed of the drum being 30 cm./hr., the actual rate of absorption in the first record, for instance, would be 0.002 c.c./min.

#### NOTE ON THE EFFECT OF INCREASED AIR PRESSURE AT THE LEAF SURFACE ON RATE OF TRANSPIRATION AND ABSORPTION.

In Fig. 6 specimen records are reproduced to show the types of record obtained when investigating the effect of pressure upon the transpiration rate of an *Aesculus* branch, the upper part of which (bearing the leaves), was enclosed in a cast iron cylinder containing compressed air at any desired pressure up to about 30 atmospheres. The air in the cylinder was prevented from becoming too humid by means of calcium chloride, and a plate glass window was provided at the top of the cylinder, through which the branch could be adequately illuminated by two 100-watt lamps. The base of the branch passed out through a specially devised seal at the bottom of the cylinder, and was connected with the vessel A shown in Fig. 1. Fuller details of these experiments will be published later in a separate communication. The records given relate to experiments in which the branch was exposed, firstly to air at atmospheric pressure in the cylinder, then to air at a higher pressure, the excess being in the first, second, and third records, five, twenty, and one hundred pounds per inch respectively, and subsequently to atmospheric pressure again.

In all cases the excess pressure was applied at the point marked by the thick vertical line, and atmospheric pressure was reinstated at the

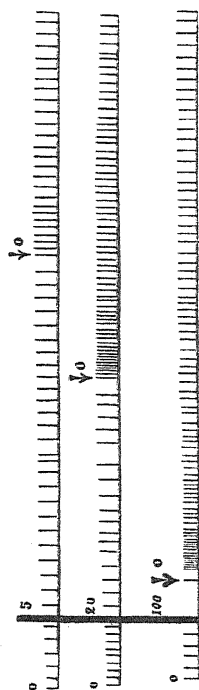


TABLE.

	Rate at atmospheric pressure,			Excess pressure applied lb./in.	Rate at excess pressure.			Subsequent rate at atmospheric pressure.			
	divs./cm.	c.c.	%.		divs./cm.	c.c.	%.	Initial.	divs./cm.	c.c.	%.
I.	4	0·002	100	5	3·46	0·00173	86·5	7	0·0035	175	100
II.	6·5	0·00325	100	20	3·46	0·00173	53·2	14	0·007	215	84·5
III.	4	0·002	100	100	0·0	0·0000	0·0	18	0·009	450	100

point marked by an arrow. It will be seen from the records and from the accompanying table in which the results are interpreted in figures, that exposure to a pressure of 5 lb./in. above atmospheric leads to a reduction in the rate of absorption to 86.5 per cent. of the original value, the rate subsequently quickening to 175 per cent. of the original rate when the pressure is released. This value then tends to fall off to the original rate. With an excess pressure of 20 lb./in. (second record) the reduction in absorption rate is down to 53.2 per cent. of the original value, and the increase on exposing again to atmospheric pressure correspondingly more pronounced, being to 215 per cent. of the original rate. With an excess pressure of 100 lb./in., as shown in the third record, absorption ceases altogether, and when the pressure is released after a short interval, absorption begins again very rapidly, at 4.5 times the original value. This rate then falls gradually back to normal. Increases in pressure are, therefore, shown always to cause a reduction in the rate of absorption. The greater the pressure the more marked is the reduction, and the more marked also is the temporary quickening of the absorption, which takes place subsequently on return to normal pressure. This quickening is evidently due to the re-expansion of compressed leaf-cells, and its extent is dependent upon the value of the excess pressure to which the cells have been exposed.

#### SUMMARY.

A recorder is described which is capable of recording absorption rates continuously in  $1/10,000$ ths of a c.c., the sensitivity being variable at will for different rates.

Absorption causes a small mercury column to be drawn backwards and forwards in a capillary tube between platinum contacts, the reversals of flow being secured by means of an electrically operated slide-valve. Each cycle of operations is electrically recorded on a drum, and marks the absorption of 0.002—0.0001 c.c.

Reliability of the slide-valve is secured by an electric method of running in with suspension of rouge in oil.

Reliability of the platinum contacts is secured by a special cut-out device, which automatically breaks the circuits elsewhere in such a way that there is no sparking at the contacts, there being no current in the circuit when the break at these contacts takes place.

The recorder may also be used as a porometer.

Some preliminary results of an investigation of the effect of pressure on transpiration in which the recorder has been used are given in the text. Further results of this investigation will be published later.

## NOTE.

**A NOTE ON EXPERIMENTS CONCERNED WITH BIOTIC FACTORS OF THE SEA-SHORE.**—Whilst working independently on the marine animal and algal communities of Lough Ine, Co. Cork, it became evident to each of us that, amongst many factors which determine their distribution, biotic factors play an important, and perhaps decisive part. Indeed, in some cases the interrelationship of plant and animal communities is the determining or master factor. Hitherto little attention has been given to this aspect of marine ecology, and after discussing our separate problems it was felt desirable to set up experiments under natural conditions, with a view to finding out the nature and influence of the biotic factors at work. The sheltered and isolated position of Lough Ine render it an almost ideal site for such experiments.

In this note we give an account of the nature of the experiments already set up, and refer to the problems upon which it is hoped they will throw some light.

1. On certain almost sheer rocky cliffs in very sheltered regions of the Lough, the tunicate *Leptoclinum* occurs in greyish colonies, forming a continuous belt about 6 in. to 8 in. in width, just below low-water mark. It frequently spreads as an epizootic covering on *Corallina* sp., *Laminaria saccharina*, and *Himanthalia lorea*. The presence of this belt causes a discontinuity in the Dictyota-Laminaria saccharina association. In order to ascertain the relative rates of growth (and therefore of colonization) of *Leptoclinum* and of Algae occupying a similar position, and also in order to find out whether the angle of slope is of importance in determining their relative distribution, a number of bare slabs of rock have been fixed at various angles and at varying depths. As these slabs extend through several algal zones, it is expected that they will provide additional evidence regarding the rate of spreading of algal communities.

2. A discontinuity or replacement phenomenon of a similar character occurs when the shore changes from vertical rocks to a region of flat stones. This change is frequently quite abrupt, and results in the sudden transition from a *Laminaria saccharina* belt to a *Paracentrotus lividus* community on the flat slabs. (This *Paracentrotus* community, which consists of *Anomia ephippium*, *Pectenvarius*, *Pomatoceros triqueter*, *Sponges*, *Polyzoa*, *Tunicates*, together with encrusting Rhodophyceae, is a well-marked feature of many parts of the shores of Lough Ine.) Clean bare slabs of different sizes have been placed at varying angles in the transition zone, and the future colonization of these will be examined from time to time. It is hoped that these slabs will show not only the rate of colonization, at differing depths and varying angles, of the plant and animal communities, but also the seasonal and transient communities which must occur before a final and apparently stable equilibrium is reached.

3. There are certain regions of the Lough, e.g. the south shore, which are very



rich in species and in individuals of both plants and animals.<sup>1</sup> Other areas are comparatively poor in this respect, and the Algae occurring in them are frequently, through lack of competition, in pure and closed associations. There are found, for instance, rock surfaces and horizontal slabs bearing copious growths of germlings of *Pelvetia canaliculata* and *Fucus spiralis*. By removing some of these slabs and carefully breaking off pieces of the rock surface, it has been possible to utilize these two Algae for an experiment of a twofold nature. Along the south shore of the Lough there is a region where a gradually shelving strand is backed by a steep cliff at high-tide level. A rough set of steps, made of large flat stones, has been constructed, thus forming a descending series from the *Fucus spiralis* zone to a belt of *Himanthalia lorea* just below low-water mark. On these steps have been placed the germling plants referred to above. Periodic examination will show :

- (a) The influence of increased submergence on their growth and development.
- (b) The effect of intensive competition on a well-established closed association growing under optimal and minimal conditions.

By way of control and for purposes of comparison a set of steps, parallel with those bearing *Pelvetia* and *Fucus spiralis*, have been constructed, the upper surface of each step being quite bare.

4. The distribution of certain Algae, e.g. *Himanthalia lorea* and *Cystoseira ericioides*, in the Lough and its approaches presents another problem. It is intended in the near future, to make observations and experiments in the hope of determining whether either of two causes, which appear to be the possible, is responsible for their appearance in the Lough. Of these one set applies to the possible rejection, for either chemical or aesthetic reasons, of the spores of certain Algae by ciliary feeding animals; the other to the buoyancy of these spores in currents of different strengths.

From its position, biological and physical features, the last including absolute freedom from contamination, Lough Ine may be described as a natural laboratory tank, and it is hoped that the solution not only to these, but also to many other problems, may be worked out along its shores.

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**A NOTE ON THE LONGEVITY OF CERTAIN SPECIES OF THE FUCACEAE.**—In the recently published 'Manx Algae', Dr. M. Knight, dealing with the problem of perennial algae, writes 'Longevity is not a well-marked feature of algal life . . . it is doubtful whether on British coasts the span of life of even these so-called perennials exceeds two or three years'. The following observations on *Fucus spiralis*, *Fucus vesiculosus*, *Fucus serratus*, and *Ascophyllum nodosum* give some indication as to the length of life of some of the larger brown algae.

<sup>1</sup> Journ. Ecology, vol. xix, no. 2, pp. 427-30 and 439-442.

In February 1926, in order to investigate the stimuli which cause the pronounced adventitious branching of many of the Fuci, a number of plants of the above species, growing along the coast of Gower, Glamorganshire, were wounded on both midrib and wing at definite distances from the holdfast. The results of the experiment were largely negative, and provided no satisfactory evidence of adventitious branching due to wounding. The plants, which varied greatly in size and luxuriance (and therefore in age), were marked by ringing the midrib near the holdfast with four or five turns of rustless bee wire, the ringing being repeated when necessary. Altogether 48 plants were marked, 12 of each species. After sixteen months 32 plants remained, as no evidence of branching could be discovered regular periodic observations were discontinued, but each time the area was subsequently visited, the plants were examined, and not until 1930 had they all disappeared. The following table summarizes the observations:

Year.	Month.	Number of plants.				Length of life.	
		<i>Fucus spiralis.</i>	<i>Fucus vesiculosus.</i>	<i>Fucus serratus.</i>	<i>Ascophyllum.</i>	Years.	Months.
1926	Feb.	12	12	12	12	—	—
"	July	12	11	12	12	—	5
"	Sept.	11	10	12	10	—	7
1927	Jan.	10	6	9	8	—	11
"	June	10	6	9	7	1	4
"	Oct.	8	6	8	6	1	8
1928	Mar.	5	3	5	2	2	1
"	Oct.	4	3	5	1	2	8
1929	Feb.	2	0	3	0	3	0
"	July	2	—	3	—	3	5
1930	Feb.	—	—	2	—	4	0
"	Apr.	—	—	1	—	4	2

The observations, though not the result of a planned experiment, are given as being of interest in connexion with the question of the longevity of individual algae, and the following general remarks may be made:

1. In all cases the plants which disappeared first were those of large size, and presumably therefore the oldest.
2. The survivors after the second year were those growing in sheltered habitats, protected by reefs or by the dip of the rock.
3. Judging by the size attained by the youngest plants during the second year of the observations, the larger plants at the commencement of the experiment were about two to two and a half years old. During 1927 many of them showed clear signs of ageing, such as the loss of the upper part of the thallus, thickening of the basal parts, especially the holdfast.
4. The most notable losses occur between October and February, and are without doubt due to the autumnal gales. Plants which survive these gales appear to be able to prolong their life throughout the spring and summer of the following year.
5. *Fucus spiralis* and *Fucus serratus* appear to be, on the average, longer-lived species than the other two.

It is probable that even under sheltered conditions the life of the larger Fuci

does not exceed four to five years, and that under conditions of moderate exposure three to four years is the average age. Unlike plants on land, death results not so much from old age as from continued buffeting and eventual detachment during stormy weather. In this the perennial marine algae stand in marked contrast with the short-lived (so-called 'annuals') species which live through their normal life-cycle from germination to death under favourable external conditions.

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**ON A SPOROCARP PROBABLY ATTACHED TO A FROND OF NEUROPTERIS SCHLEHANI, STUR.**—A specimen of *Neuropteris schlehani*, Stur., with a seed-like body probably attached to the frond was discovered by Mr. W. P. Hedley at the High Shilford Colliery, near Riding Mill, about three miles south-east of Corbridge-on-Tyne, Northumberland. The only seam worked at this colliery is known as the High Shilford Coal, one of the coals of the Nafferton Coal Group which occur in the Millstone Grit of that area.<sup>1</sup> The writer is indebted to Mr. R. G. Carruthers, Mr. G. A. Burnett, and to Mr. W. P. Hedley for information concerning the horizon of this seam.

The roof of the High Shilford coal consists of dark-grey, rather fine-grained shale which is often unevenly bedded and contains abundant plants in certain bands. The most common species is *Neuropteris schlehani*, Stur., which is usually represented by numbers of isolated pinnules, but occasionally complete fronds can be found. Associated with *N. schlehani* are the following plants: *Neuropteris* sp., *Sphenopteris obtusiloba*, Brongt., *Calymmatotheca (Crossotheca) hoeninghausi*, Brongt., *Calymmatotheca stangeri*, Stur., *Eupecopteris minor*, Kidston, *Peopteris* cf. *aspera*, Brongt., *Dactylothea plumosa* (Artis), *Sphenophyllum* sp. nov., *Calamites undulatus*, Sternb., *C. suckowi*, Brongt., *C.* sp., *Asterophyllites longifolius*, Brongt., *Cordaites principalis* (Germar), and *Lepidodendron* cf. *obovatum*, Sternb.

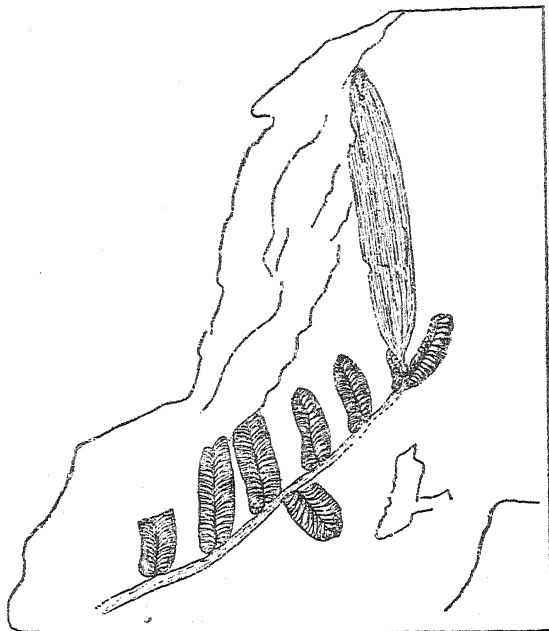
A similar assemblage of plants is known to occur in the upper part of the Millstone Grit Series and in the lower part of the Coal Measures of South Wales and of other areas.

The specimen consists of a frond of *Neuropteris schlehani*, Stur., including the rachis, five well-preserved pinnules, and three fragmentary pinnules. A 'seed-like' body appears to be attached to the rachis at the apex of the frond. The frond and seed-like body are preserved as impressions on the shale.

The seed-like body is 25 mm. long and 4.5 mm. broad. It is elongately elliptical in shape, tapering at the apex and base. It joins the rachis between the two apical pinnules. The surface of this structure is marked by numerous, close, parallel, longitudinal striae. There are also traces of several ribs, but they are not well marked.

<sup>1</sup> Hedley, W. P., The Geology of Northumberland and Durham. III. The Lower Carboniferous. Proc. Geol. Assoc., xiii. 238, 1931.

At first the writer thought that this seed-like structure represented the true seed of *Neuropteris schlehani*, Stur. Later, she was impressed by the resemblance of this form to certain species of the genus *Rhabdocarpus*. In certain characters this seed-like body resembles *Rhabdocarpus elongatus* Kidston, in which Mr. Hem-



*Neuropteris schlehani*. Stur., with a seed sporocarp which appears to be attached to the frond, High Shilford Coal, High Shilford Colliery, near Riding Mill, Northumberland.

mingway found a number of isolated spores in specimens from Dudley.<sup>1</sup> Prof. Halle of Stockholm has spent much time in investigating specimens of supposed seeds from Carboniferous Rocks. He has come to the conclusion that *Holcospermum* (*Rhabdocarpus*) *elongatum*, though seed-like is similar in structure to *Whittleseya elegans*. The 'supposed seed' consists of about 8 to 9 sporangia which are filled with spores. The sporangia are concrescent and form a synangium'.<sup>2</sup>

More recently Mr. W. P. Hedley succeeded in finding five unattached specimens of this seed-like body from the same horizon and locality. All these specimens are similar in appearance to the above one described, but three of the five specimens showed a 'V'-shaped opening at the presumed apical end.

The writer made a number of canada balsam transfer preparations, and in each of the five specimens a number of isolated spores can be seen. The spores vary in size from 0.015 to 0.174 mm. A few possess rather thick outer coats. The scarcity of the spores in these structures can be explained, if we regard

<sup>1</sup> Halle, T. G., The Morphology of *Whittleseya* and Related Forms. Report Proc. Fifth International Botanical Congress, Cambridge (1930), 473, 1931.

<sup>2</sup> Ibid., 473.

these seed-like structures as ripe, more or less empty spore capsules. In support of this conclusion it should be noted that the distinct V seen in three of the specimens may possibly indicate the point of dehiscence of the spore capsule. From the material examined it is impossible to interpret the internal structure of these spore-bearing organs. The traces of ribs may represent the common walls of the sporangia, if they are united together in the form of a synangium as in the case of *Holcospermum* (*Rhabdocarpus*) *elongatum*, described by Prof. Halle.<sup>1</sup> Prof. Halle recently sent the writer a drawing of *Holcospermum elongatum*, showing the arrangement of the sporangia in the synangium, and for this structure Prof. Halle proposes a new generic name.

Prof. Renier stated<sup>2</sup> that in 1914 he 'was about to describe a kind of *Whittleseya* or *Neurospermum* found in association with *Neuropteris schlehani*, and first announced by him to be the seed of that species'. Later Prof. Renier altered his opinion, because he found specimens showing long pedicles opposite to the fringed border, and also because, associated with these bodies, was a kind of seed related to *Rhabdocarpus*, of which three specimens were found attached together. It is difficult to interpret Prof. Renier's specimens in view of the present evidence.

Dr. W. J. Jongmans suggested<sup>3</sup> that several of the seeds found attached to various forms belonging to the Pteridosperms may possibly represent male organs, e.g. *Neuropteris obliqua*, *N. heterophylla* and *Alethopteris valida*, and he stated that it was regrettable that Prof. Halle had not been able to find spores in the seed of *Neuropteris obliqua*.

Although it appears that no instance of seeds attached to fronds of *Neuropteris schlehani* has been recorded, both Prof. Renier<sup>4</sup> and Prof. Bertrand<sup>5</sup> have recorded the occurrence of oval ribbed seeds with this species. Prof. Renier referred the seeds to the species *Rhabdocarpus* cf. *unicatus* Berger, and Prof. Bertrand stated that his seeds were identical. Prof. Bertrand and Arber<sup>6</sup> independently proposed the generic name *Neurospermum* for the seeds of *Neuropteris heterophylla* and *N. obliqua* in preference to *Rhabdocarpus*,<sup>7</sup> and Prof. Bertrand referred the seeds associated with *N. schlehani* to *Neurospermum schlehani*. Prof. Seward<sup>8</sup> stated that the 'generic term *Neuropterocarpus* used by Grand'Eury in 1904, though not defined by him, has priority, and avoids the adoption of a new designation for the seeds attached to *Neuropteris* fronds'.

<sup>1</sup> Ibid., 473.

<sup>2</sup> Renier, A., Discussion on the Position of the Pteridosperms in the Plant Kingdom and their Relation to Ferns. Ibid., 483, 1931.

<sup>3</sup> Jongmans, W. J., Discussion on the Position of the Pteridosperms in the Plant Kingdom and their Relation to Ferns. Ibid., 483, 1931.

<sup>4</sup> Renier, A., Sur une graine qui paraît devoir être rapportée à *Neuropteris Schlehani* Stur. Ann. Soc. Scient. Bruxelles, 27 Oct. 1910.

<sup>5</sup> Bertrand, P., Les Fructifications de Néoptéridées recueillies dans le terrain houiller du Nord de la France. Ann. Soc. Geol. Nord, xlii, 113, 1913.

<sup>6</sup> Arber, E. A. N., A Revision of the Seed Impressions of the British Coal Measures. Ann. Bot., xxviii, 81, 1914.

<sup>7</sup> Seward, A. C., Fossil Plants, iii. Pteridospermeae, Cycadofilices, Cordaitales Cycadophyta. Camb. Univ. Press, 1917.

<sup>8</sup> Ibid., 116.

It should be pointed out that the sporocarp figured in this paper is much more elongated than *Rhabdocarpus* cf. *tunicatus* of Renier and *Neurospermum schlehani* of Bertrand, although it agrees with the latter forms in having a fibrous surface. The writer has examined some specimens of *Rhabdocarpus* associated with *N. schlehani* from the Continent, in the Kidston Collection; and it is extremely probable that the seeds described by Renier and Bertrand represent sporocarps.

Mr. W. D. Ware, who has assisted the writer in making extensive collections of fossil plants from the Millstone Grit of South Wales, recently discovered an interesting, although poorly-preserved specimen of *Neuropteris* from near the base of the Millstone Grit Series at Penwyllt, South Wales. The specimen is interesting because it shows what appears to be a sporocarp similar to the one described in this paper, in a terminal position, although not attached to the frond.

There is remarkable variation in the form and size of the plant *Neuropteris schlehani*, and it is probable that several forms are included in this species, some with much more elongated sporocarps than others. Dr. Jongmans informed the writer that he regards certain variations in the frond of *Neuropteris schlehani* as stages in the evolution of this species. The writer is at present investigating the variation in form and the vertical range of this important zonal plant in various parts of Great Britain.

The occurrence of spores in a sporocarp attached to the frond of *Neuropteris schlehani*, Stur., raises a question concerning the affinities of the genus *Neuropteris*. The writer has found *Trigonocarpus* associated with fronds of *N. schlehani*, and it is suggested therefore that the sporocarp contained microspores. In the writer's opinion the genus *Neuropteris* includes a number of groups, e.g. *N. schlehani* group, which is closely related to *Alethopteris*, *N. obliqua* group, *N. heterophylla* group, and the *N. scheuchzeri* group &c.

The specimen is preserved in the collection of the British Museum (Natural History).

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**THE TRANSPIRATION OF THE SPOROPHYTE OF POLYTRICHUM COMMUNE.**—In a recent paper ('Absorption and Conduction of Water and Transpiration in *Polytrichum commune*', Ann. Bot., xlii, 1932) the writer recorded the results of transpiration experiments with the leafy stems of *Polytrichum commune*, showing that these plants, both under laboratory conditions and growing *in situ*, lose a considerable amount of water which ascends through the central strand of the axis. During the early part of the summer of this year, opportunity was taken to extend the work to the sporophyte of the same species, and in view of a fuller account, including anatomical details of the absorbing foot which it is hoped to publish later, it may be useful to record briefly some experimental results already obtained. Weight potometers were used of the same design as those described in the paper cited. Each potometer held a single sporophyte attached to the parent stem from which the leaves had been removed. The capsules had attained their full size but were still

green, and the stomata, restricted to the narrow ring immediately above the stomatal physis, were fully developed. The potometers were weighed daily, and apart from very slight variations, probably due to changes in temperature, the transpiration rate remained remarkably steady over a period of more than three weeks. The total loss in weight for three potometers during a period of twenty-one days was 0.14 gm.

A second series of potometers, containing sporophytes in which the stomatal ring was covered with vaseline showed, on the average, a loss in weight of 0.051 gm. in twenty-one days, approximately one-third of the loss in untreated sporophytes.

Experiments were also carried out to ascertain what part, if any, is played by the stomata, with special reference to stomatal movement, but this aspect of the problem requires further investigation which it is hoped will be undertaken next year.

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*July, 1932.*





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